Doctoral (Ph.D.) Dissertation

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Study of health promoting components throughout the malting and brewing process

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LIST OF ABBREVIATIONS

ABTS - 2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)

CUPRAC - cupric ion reducing antioxidant capacity

DAD – Diode Array Detector

DPPH - diphenyl-1-picrylhydrazyl

EBC - European Brewery Convention

ET - electron transfer

FCR - Folin-Ciocalteu reagent

FRAP - ferric ion reducing antioxidant power

GI - gastrointestinal

HAT - hydrogen atom transfer

HPLC - high performance liquid chromatography

IBU - international bitterness units

IPA – India Pale Ale

MRP - Maillard reaction products

PVPP - polyvinylpolypyrrolidone

ROS - reactive oxygen species

TEAC - Trolox equivalent antioxidant capacity

TFC - total flavonoid content

TPC - total polyphenol content

TPTZ - 2,4,6-tripyridyl-s-triazine

1. INTRODUCTION

In recent years, the lifestyle of people, especially in western countries, has changed, we are pursuing an accelerated way of life, which also affects our eating habits. Processed foods make up an increasing proportion of the food consumed. As a result of processing, they lose their nutritional value, primarily their biologically active nutrients. Intake of biologically active nutrients such as vitamins and antioxidants are essential for the proper functioning of the body. To know how we can meet our body's need for vitamins and antioxidants, we also need to know how the processing of raw materials during food production affects these components. My dissertation is intended to help answer this question.

It is assumed that on a population level, nutritional requirements for folate cannot be completely covered by a varied diet, as recommended by national health authorities. Dietary intake is below recommendations in numerous countries, including Hungary, owing to low consumption of folate-rich foods, e.g., pulses, citrus fruits, and leafy vegetables (OHRVIK et al. 2011, OTÁP 2014).

Cereals are considered a good source of folate. There are publications dealing with the folate content of different cereals (oat, wheat, barley, rye) but with malting the folate content of cereals can be further increased. Nonetheless, there is only one scientific publication investigating the evolution of folate content during malting of barley which was authored by WALKER (2003). The effect of pilot-scale malting of different barley cultivars was studied on folate content. The comparison of the effect of industrial and pilot-scale malting on folate content has not been studied. The comparison of industrial and pilot scale would be a practical use because the great majority of malted products that are used as food ingredients are results of industrial malting. Furthermore, the effect of roasting on folate content of barley malt has not been studied, though special malts, which are treated at higher temperatures are also used widely for human consumption in the form of brewing malt, malted syrups, colorant in bakery products, or coffee substitute.

There are numerous scientific publications about the folate content of several types of beers available in commercial trade, in these it has been reported that beer can be a valuable source of folate depending on the beer type, but a considerable deviation is observed between the different products. There is a lack of information about the effect of raw materials and technological steps of brewing on folate content that could be helpful to understand the evolution of folate during the brewing process and to get a deeper insight into what makes the folate content of each product so different.

In addition to folate, the intake of antioxidants is also essential. Nowadays, we are exposed to several external physical and internal psychological stress effects. Stress influences the balance of antioxidants and free radicals in our body, which can lead to various diseases. To maintain this balance, we need to consume foods rich in antioxidants, primarily foods of plant origin. However, it is shown that we do not consume enough.

Supplementation with cereals, malt beverages and alcohol-free beer can be a good alternative to this. In addition to the health effects, antioxidants and phenolic compounds are also very important from the technological point of view in brewing. There are studies in the literature that examine antioxidants and polyphenols during malting or brewing, or in different types of beers, but a comprehensive study that gives a detailed picture of these compounds from the grain to the finished product is not available.

Adjuncts and special malts are widely used in the brewing industry globally, and extensive knowledge is available on their application technology. Some studies are currently investigating the phenolic composition of different types of grains, but there is little available information on their effect on wort phenolic composition when used as brewing adjuncts. In addition, there is no literature on the effect of special malts, high gravity brewing, infusion or decoction mashing on the phenolic composition of the wort.

2. OBJECTIVES TO ACHIEVE

The aim of my dissertation is to form a comprehensive picture about the evolution of folate content, antioxidant activity and phenolic content from the raw materials throughout the malting and brewing process until the final beer. To achieve this, I set the following goals:

- introduce and process the available literature

Folate experiments:

- determine the folate content of malts produced from different grains
- determine the folate content of different barley varieties
- determine the folate content of barley malts produced on pilot and industrial scale
- investigate the evolution of folate content during pilot and industrial scale malting
- investigate the effect of roasting on the folate content of barley malts
- investigate the effect of an extended protease rest on folate content
- investigate the evolution of folate content during laboratory and pilot scale brewing

Total phenolic composition and antioxidant activity experiments:

- determine the total phenolic composition and antioxidant activity of malts produced from different grains
- determine the total phenolic composition and antioxidant activity of different barley varieties
- determine the total phenolic composition and antioxidant activity of barley malts
- investigate the evolution of total phenolic composition and antioxidant activity during pilot and industrial scale malting
- investigate the effect of roasting on total phenolic composition and antioxidant activity
- determine the total phenolic composition and antioxidant activity of different hop varieties
- investigate the evolution of total phenolic composition and antioxidant activity during laboratory and pilot scale brewing

Targeted phenolic compounds, total flavonoid content, total polyphenol content, and DPPH radical scavenging activity experiments:

- investigate the effect of adjuncts and special malts on targeted phenolic compounds, total flavonoid content, total polyphenol content, and DPPH radical scavenging activity of congress worts

- compare the effect of infusion and decoction mashing on targeted phenolic compounds, total flavonoid content, total polyphenol content, and DPPH radical scavenging activity
- investigate the effect of high gravity brewing on targeted phenolic compounds, total flavonoid content, and total polyphenol content

3. OVERVIEW OF LITERATURE

Malting and brewing are centuries old technologies that may seem simple yet very complex processes. The raw materials and parameters used in each technological step affect the later stages of the process and the chemical properties and quality of the final product. The overview of literature is written in the order of the technological processes and only deals with ingredients and processes which are relevant to the topic of the dissertation.

3.1 Barley

Barley malt is the main ingredient of brewing besides water and hops. Smaller amounts of malt are produced from wheat, rye, triticale, sorghum, millets, oats, maize, or rice but these are used only to brew special types of beers. Barley malt is used in the vast majority (BRIGGS 1998).

Barley (*Hordeum vulgare*) is a highly adaptable cereal grain that has the ability to grow in adverse conditions (WUNDERLICH et al. 2011). Regions with moderate temperature and sufficient precipitation are the most suitable for production of good quality malting barley (PRŽULJ et al. 1997). Barley is one of the most cultivated crops globally (12% of total cereal cultivated), ranking fourth among cereal grains after wheat, rice, and maize. Approximately 65% of cultivated barley is used for animal feed, 33% for malting, whereas only 2% is used directly for human consumption (IDEHEN et al. 2017). Typical food applications of barley: pearl barley is used in soups and dressing flour; milled barley is used in baby food, bread, biscuits; malted barley is used for brewing, baking, and producing distiller's alcohols, or in milk-based beverages, malt syrups, coffee substitute and so on (VASAN et al. 2014).

Barley cultivars can be classified as spring or winter varieties. In Europe, winter barley is usually sown in October, and spring barley in March (PRŽULJ et al. 1997). Traditionally, spring barley cultivars have been used for malting, but there is much potential for winter malting varieties. The advantages of winter over spring barley include increased yield, and earlier maturation before arrival of high temperatures and water stress, therefore decreasing the irrigation requirements (MUÑOZ-AMATRIAÍN et al. 2010). Late sowing of spring barley reduces the yield and increases the protein content (LE BAIL et al. 2003). PRŽULJ et al. (1997) reported that winter barley has better malt quality and grain characteristics, especially during periods of drought. Winter barley has therefore been more widely grown in the recent years as the temperature increases and the rainfall declines due to climate change.

Knowledge of the **structure of the barley grain** is essential to understand the processes that take place during malting. The barley kernel has a light-yellow grain color. The germ is

comprised mostly of lipids, and the endosperm contains starch granules embedded in a protein matrix. Several layers, namely the aleurone, testa, pericarp, and outer husk, surround them (Figure 1). These outer layers are rich in polyphenols and cellulose (VAN DONKELAAR et al. 2015). Hulled and hull-less barley are the two major types cultivated, however hulled barley is preferred for brewing as the husk contributes to beer's flavor and acts as a filter layer during wort separation. The husk is also important because it protects the growing ascospire during germination. Barley can be two-row or six-row, which refers to how the grains are arranged around the stalk. European brewers prefer two-row barley which has a higher starch content and lower protein content compared to six-row barley (MUÑOZ-AMATRIAÍN et al. 2010). Two-row barley produces malt with high extract, lighter color, and lower enzyme content (GUPTA et al. 2010).

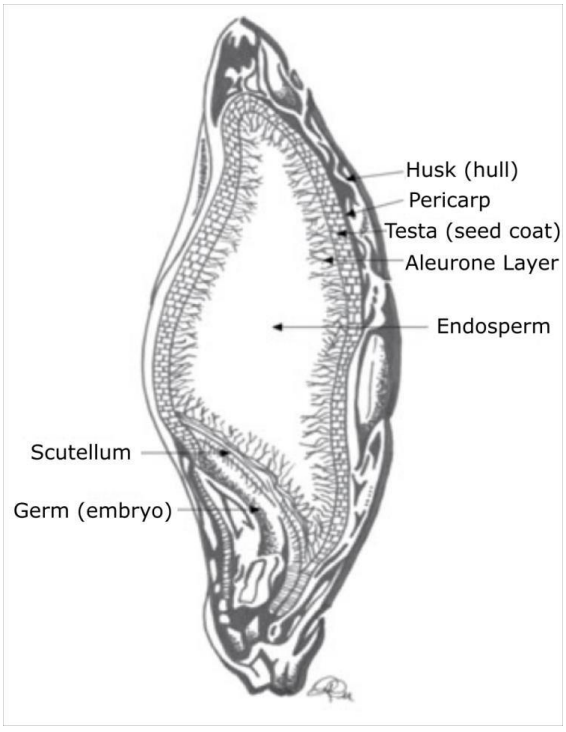


Figure 1: Barley seed cross section (MC KEY et al. 2011)

3.2 Malting

Malting is the process of converting barley grains to be suitable for brewing. Malt was originally only made in winter months, from November to April in Europe. This was because low temperatures were needed to control the germination process. In the 19th century, the industrialization of malting began as larger breweries were constructed. In the past, breweries had malt houses on their premises, but now most breweries purchase malt from independent

maltsters due to which 90% of malt produced from malting barley is purchased by brewers (MEUSSDOERFFER 2009). ZHOU (2010) reported that in the United States, 80% of the malt is used by the brewing industry, 14% for distilled alcohol production and the remainder in malt syrup, malted milk and breakfast cereals.

Malts serve as sources of soluble extract, enzymes, color, and character for the products made from them (BRIGGS 1998). Malt quality depends on both the barley quality and the malting process. Many characteristics determine malting quality, including protein content, malt extract, diastatic power, alpha-amylase activity and β-glucan content (MUÑOZ-AMATRIAÍN et al. 2010). The malting process consists of three main steps: the first is steeping to increase the moisture content of the kernels, the second is a controlled germination to ensure given physical and biochemical changes, which are then stabilized by the final step, kilning. Germination and kilning have been found to change the appearance, flavor, and taste of the grain as well as their nutritional value (HÜBNER et al. 2013).

During malting, barley kernels undergo an incomplete natural germination process that involves a series of enzyme degradations of the barley kernel endosperm (GUPTA et al. 2010). Dormancy retards germination, and an appropriate storage time after harvest helps remove dormancy and improve germination characteristics. Storage conditions are very important. The barley should be stored in cool and dry conditions, as warm or humid conditions will decrease the germinative capacity (WOONTON et al. 2005). The fundamental process of malting has remained unchanged for many years, and the entire malting process usually takes around six days to complete (GUIDO et al. 2014). There are several **objectives of malting**. These include hydrolysis of the endosperm cell walls (predominantly made up of β-glucan); hydrolysis of a portion of endosperm proteins; production of enzymes, such as alpha-amylase, in the kernel which will be further utilized during brewing; and to develop a desirable malt color and flavor (WOONTON et al. 2005).

The main steps of the malting process are showed in Figure 2. The first step of the malting process is **steeping**, where the grains are immersed in water. They begin to take up water, and the increase in moisture content induces the germination process to begin. Steeping consists of alternating periods of water immersion and air rest periods. Oxygen supply is essential at this step. The barley embryo uses oxygen dissolved in the water and in the air for respiratory purposes (GUIDO et al. 2014). BRIGGS et al. (2004) reported that the optimal temperature for steeping is 12°C. The uptake of water during steeping causes the grain to swell up to 1.4 times its original volume. Steeping also removes film of dirt and microorganisms from the grain, and this removal is promoted by agitation during steeping (GUIDO et al. 2014).

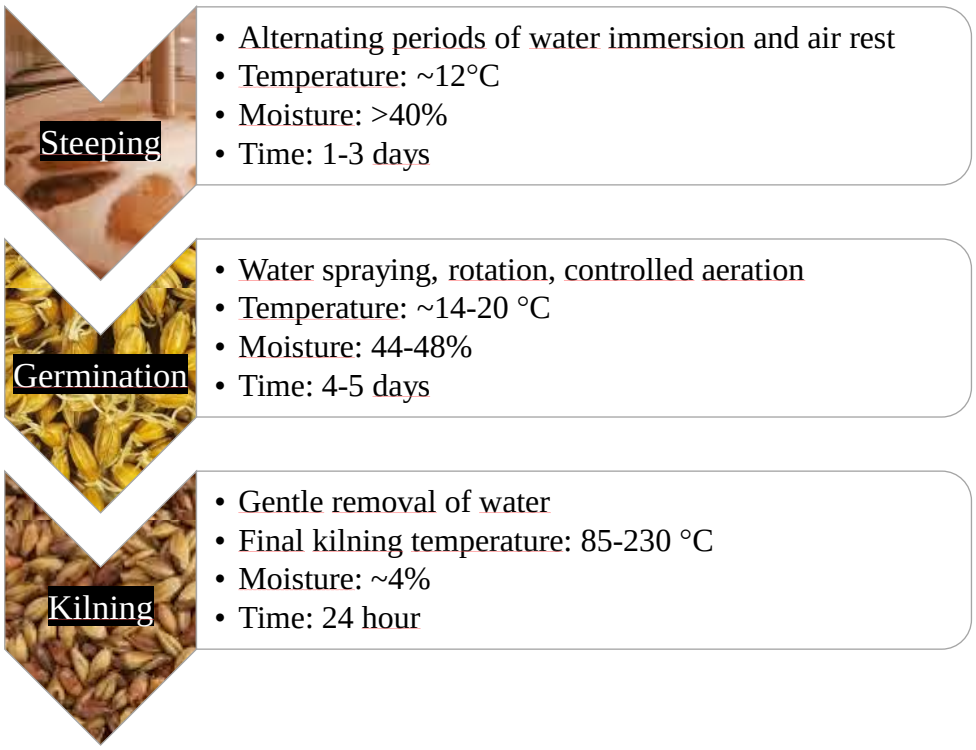


Figure 2: Malting process

The second step of malting is **germination**. This step traditionally occurs under humid and ventilated conditions over four to six days at 14-20°C. The grains are rotated to ensure homogenous kernel germination (GUIDO et al. 2014). The root sheath protruding from the base of the grain indicates the start of germination. The grain grows protruding rootlets from the germ at its base and the ascospire grows beneath the husk (BRIGGS et al. 2004). The production and release of several hydrolytic enzymes occurs during germination. The embryo releases gibberellic hormones, which initiates the formation of enzymes in the aleurone layer. These enzymes are released into the starchy endosperm (BRIGGS et al. 2004). The enzymes produced include alpha-amylase, endo-peptidase and limit dextrinase, while beta-amylase is already present, so it is only activated during germination. The endosperm cell walls are degraded as β-glucans and pentosans are broken down. Starch and protein in the endosperm are also degraded (GUIDO et al. 2014). The chemical composition of the barley kernel is modified due to this enzyme activity. VAN DONKELAAR et al. (2015) reported that there is an 18% decrease in the starch content, as the starch is broken down into simple sugars. The polyphenols increase two-fold and there is an 80% reduction of β-glucans.

The product of germination is green malt (BRIGGS et al. 2004). To produce malt, the third and final step of the malting process, **kilning**, must be done. Kilning dries out the malt and prevents

further kernel growth and modification. Green malt should be dried gently by slowly increasing air temperature so as not to denature the enzymes required later in the brewing process. The final drying air temperature is around 80-85°C in case of pale malts, and the final moisture content of the malt is around 4% (BRIGGS 1998). Kilning also removes undesirable flavors. The dried malt is stable and can be stored for long periods of time. It is also friable and readily crushed and milled due to its soft endosperm (GUIDO et al. 2014).

3.3 Malt types

In recent years market trends and consumer demands for different beer types have led maltsters to produce a wide range of different special malts with different colors and aromas (GUIDO et al. 2014). Malt types are classified into two main categories – malts rich in enzymes, which make up the majority proportion of the grist, and special malts, which make up a small proportion of the grist. The malts rich in enzymes include pale malts kilned at lower temperatures, while special malts are kilned at higher temperatures. Many pale malts are cured at around 80 °C, which results in very little color formation, on the other hand some special malts are roasted above 200 °C, which can be used to create a special color and taste. (BRIGGS et al. 2004)

Pilsner malt is the main ingredient to produce most beers. Some beers are brewed only using Pilsner malt, but some beers also contain a relatively small amount of special malt(s) in addition to the Pilsner malt (COGHE et al. 2006). Different types of malts are shown on Figure 3. Pilsner malt is kilned at low temperature and the final color is usually 2.5-3.5 EBC (European Brewery Convention) units. Pale Ale malt has more characteristic flavors as the finishing temperature is slightly higher at 95-105 °C. Vienna malt is kilned to 90 °C and the color is usually 5.5-6.0 EBC. It is used to make golden lagers. Munich malt is relatively dark at 15-25 EBC. Kilning of Munich malt is finished at 100-105 °C but still rich in enzymes (BRIGGS et al. 2004).

Special malts also play important roles in brewing. Special malts are applied to produce beers with different organoleptic qualities (color, taste, body, aroma). Through their use it is possible to produce infinite variations in beers. Special malts are finished on roasting drums at high temperatures and provide beer with specific desired colors, flavors, and aromas. These special malts are exposed to higher temperatures than malts in the high-enzyme category. These elevated temperatures cause intense non-enzymatic browning from Maillard reactions. Depending on the time and temperature of the thermal treatment applied, malts with a wide range of colors can be produced, from pale yellow, amber, brown, and black (COGHE et al. 2006). These Maillard reactions induce not only color changes, but also flavor-active volatiles. They are usually used

for brewing as soon as possible and not stored for long periods of time, because the desired aromas and flavors degrade during storage. A wide range of different colored caramel and crystal malts are produced. To produce these malts, green malt is stewed and gelatinization, liquefaction and saccharification take place in the kernel, which is then dried by roasting. Caramel and crystal malt have rich, characteristic flavors from caramelization and Maillard reactions that occur during roasting, and they provide mouth-filling properties. These kinds of malts offer a wide range of colors from amber to red. They impart rich and delicious caramel-like flavors and give body to beers. They are believed to improve beer stability and are often used between 10-30% of the grain bill.

Coloring malts undergo intense heating in a roasting drum after malting and a vast range of colors can be produced. No enzymes survive this heating process. Amber malts are heated up to 170°C and the final color is 40-85 EBC. Chocolate malt is heated up to 215°C and have a color usually of 900-1100 EBC. Black malt is heated up to 225°C and the final color can be 1200-1400 EBC. At this temperature pyrolysis occurs. These malts have a color from brown to black and give beer a dry, burnt, coffee like taste and dark color. They are often used at under 5% of the grain bill. (BRIGGS et al. 2004, SHELLHAMMER 2009)

Figure 3: Different types of malts

3.4 Adjuncts

Due to the competitiveness of the beer market, breweries are constantly under pressure to produce consistently high-quality beer at a lower cost. To achieve this goal, brewers have increasingly replaced malt with various less expensive adjuncts. It is estimated that up to 85-90% of beer worldwide is now produced with adjuncts (BOGDAN et al. 2017). In European countries, generally 10-30% of malt is replaced by unmalted materials, in the United States and Australia 40-50% or more, while in Africa the Figure stands at 50-75% (ANNEMÜLLER et al.

2013). The most commonly used adjunct materials are corn (46% of total adjunct), rice (31%), barley (1%), wheat (>1%) and sugars and syrups (22%) (STEWART 2016).

Brewing with adjuncts has some **advantages**, using adjuncts with a lower protein content (corn or rice) usually results in beers with improved colloidal stability. A beneficial decrease in flavor staling precursors in beer is another result of adjunct use, due to lower nitrogen levels. By altering the sugar and amino acid profile that results from the use of unmalted raw materials, changes can be made to the flavor profiles of beers. (BOGDAN et al. 2017) Wheat and barley adjuncts that have a higher protein content are reported to have foam enhancing or foam stabilizing properties (LLOYD 1986). Furthermore, using adjuncts can reduce processing costs by yielding a less expensive extract than malt and can also help save energy and water by removing the need for the malting processes. Therefore, the carbon footprint of beer production may be diminished (GOODE et al. 2006, POREDA et al. 2014; BOGDAN et al. 2017).
On the other hand, there are some **disadvantages** of adjuncts. Low levels of soluble proteins in the case of rice and corn, can result in a less full-bodied beer and lower head retention (TAYLOR et al. 2013). Some cereals, notably barley and wheat can cause a wort viscosity problem, principally derived from beta-glucans in barley and pentosans in wheat, which adversely affect beer filtration rates (LLOYD 1986, WEST 2012, FALTERMAIER et al. 2014). The absence of husks in many adjuncts can be another problem, limiting their use if the wort is to be separated in the lauter tun. A risk when using mash vessel adjuncts is that there may be insufficient quantities of enzymes from the malt, which can give rise to aggregates of cell wall constituents, finely divided protein, lipid, and undigested small starch granules (BOGDAN et al. 2017). There may also be insufficient quantities of enzymes to break down proteins and ensure an appropriate level of assimilable nitrogenous compounds available for the yeast, which can have a negative influence on beer fermentation (PIDDOCKE et al. 2011, STEWART et al. 2013).

3.5 Hops

Hops were not always an ingredient for beer, medieval beer, for which hops had not yet been added, rapidly went sour and turned into malt vinegar. Therefore, many herbs were used in attempts to prolong the shelf-life but only the hop, *Humulus lupulus* L., is used in large-scale brewing today. Hop belongs to the *Cannabinaceae* family, it is a perennial climbing plant. The hop is dioecious, male, and female flowers are produced on different plants. The male flowers produce pollen which can be carried long distances by the wind so any female plant in the

vicinity will be fertilized and produce seeds at the base of the bracteoles. For brewing unfertilized female plants are cultivated, which develop cones from the flowers.

The yellow-green, sticky in fresh conditions, cup-like glands (lupulin glands) located on the inner and outer bracteoles of the hop umbel are the greatest interest to the brewers since these contain the technically important bitter resins and aroma substances (Figure 4). The cones consist of a central strig with bracts and bracteoles attached. Most of the lupulin glands are formed on the base of the bracteoles but they are readily detached and adhere to the bracts, strig and seed. The lupulin glands can contain as much as 57% of α-acids, which are isomerized during the hop boiling process of brewing, giving beer bitterness.

Most of the **brewing value of the hop** is found in the resins and essential oils which are only slightly soluble in water. The bitter resins give the beer the fine pleasant hop bitterness, aid the salubriousness, stabilize the foam, and increase the biological shelf-life with their antibacterial properties. During thermal isomerization (wort boiling/hop boiling), five-ring structures are formed from the optical active hexa-cyclic substances. As a result of the high oxygen sensitivity of hops, numerous oxidized derivates are formed. These non-volatile, water-soluble, often complex substances dissolve in the wort and can reach the beer. The iso-α-acids and derivatives show the greatest bitterness potential. (KROTTENTHALER 2009)

Hops produce up to 3% of essential oil which is responsible for the pleasant hoppy aroma of beer. It is produced in the lupulin glands along with the resins, mostly after resin synthesis is finished. The composition of the essential oil depends on genetic (cultivar) and cultural factors but since most commercial hops are picked at an equivalent degree of ripeness varietal factors will dominate. Essential oils are volatile in steam, so most essential oil will be lost when hops are boiled in wort in a copper open to the atmosphere. To add hop aroma to the beer brewers either add a portion of choice hops towards the end of the boil or add dry hops to the beer either in cask, conditioning tank or using a dry-hopping equipment. (BRIGGS et al. 2004)

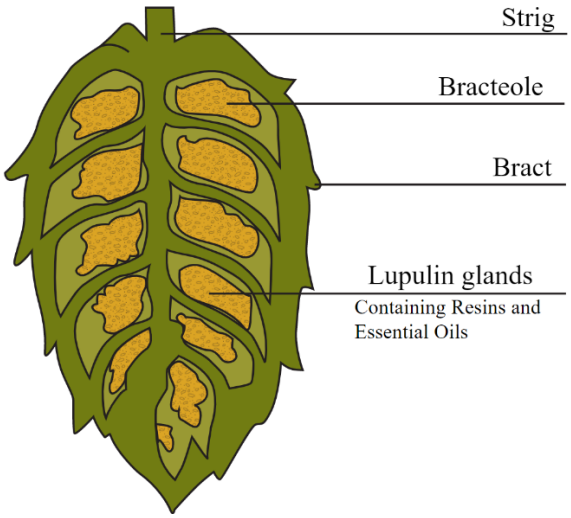


Figure 4: Cross section of a hop cone

3.6 Brewing technology

Brewing is a complex biotechnological process that has a long tradition. However, in addition to knowledge of traditions, deep biological, chemical, physical, biochemical, and technical knowledge is required to understand each of its steps. In the next chapter, I will describe the process of brewing from the crushing of the malt to the bottling of the final product. The first part of the process can be seen on Figure 5.

The brewing technology starts with the **crushing** of malted grain to expose the starchy endosperm. The resulting grist consists of broken pieces of grain and husks, the crushed endosperm can be as fine as powder to make the starch readily available by the enzymes during mashing, but the husk should retain its original form and shape to function as the filter layer later during wort separation.

After crushing the malt grist and pre-treated, tempered water is mixed and added to the mash tun, giving us the mash. The **mashing** process is the solubilization of malt components by enzymatic, physical, and chemical solution processes. In the mashing step, enzymes synthesized and activated during the malting process convert the macromolecules (starch, protein, beta-glucan etc.) to smaller particles (sugars, amino acids etc.). The goal of mashing is to break down large starch molecules into sugars which can be digested by yeast. (BRIGGS et al. 2004)

The mashing process can involve several temperature rests depending on what process the brewer wishes to accomplish. A rest is a period of time in which the mash is held at a specific temperature. In mashing, the enzymes of the malt become reactivated. Once hydrated and at the correct temperature, the enzymes will work and convert the starch into fermentable and non-fermentable sugars. They also decompose the proteins and other biological structures holding the starch in the endosperm and release it into the water. They convert the proteins into smaller

pieces and individual amino acids, and also help lower the pH of the overall system. The fermentable sugars are necessary for the fermentation step of the brewing process. These sugars include maltose, glucose, maltotriose, and a host of others that can be consumed by yeast during fermentation. While fermentable sugars are the primary product of the mashing process, mashing also creates non-fermentable sugars. These sugars are not consumed by yeast during fermentation and remain essentially unchanged by the end of the entire process. They remain in the beer after fermentation to lend body and a sweet taste. Fermentable sugars and amino acids are not only nutrients to the yeast, but also the basic compounds of the Maillard reaction that takes place during hop boiling. (KROTTENTHALER et al. 2009)

The enzymatic breakdown during mashing can be controlled by the parameters of temperature, viscosity, pH value and time. Over the course of time many different mashing processes have been developed, which can in general be divided into infusion and decoction methods. During infusion technology the temperature of the whole amount of mash is constantly raised, keeping enzymatic rests at certain temperatures. The other is decoction technology where a part of the mash is separated and boiled, thereafter added back to the other part. It can be repeated once, twice, or even three times, based on this it is called single, double, or triple decoction, respectively. The choice of infusion (enzymatic digestion) or decoction (enzymatic and physical digestion) is an important mash parameter. The partial mashes lead already during the mashing process to an increased thermal stress and an increased protein precipitation. Not only is the thermal disintegration of inaccessible structures in the endosperm important, but decoction also allows brewers to create new combinations of enzymatic rests, features not offered by infusion. (SACHER et al. 2016)

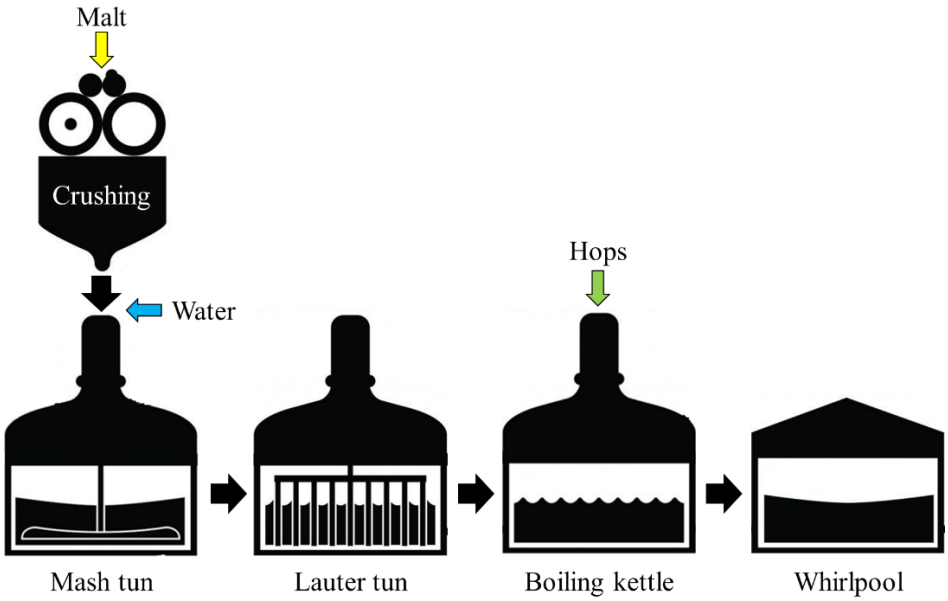


Figure 5: Brewing process part I.

After mashing the liquid part, containing the dissolved extract content, need to be separated from the insoluble solid parts (mainly the husk). This separation is called **lautering** and can be separated to two phases, one is first wort separation. In this phase, the liquid part of the mash (wort) is separated from the solid part. After first wort separation, the filter cake is washed through with hot water to dissolve the rest of the water-soluble compounds, this step is called sparging. Owing to the danger of washing out iodine-reactive β-glucans, the sparge water should have a maximum temperature of 78 °C. In order to avoid channeling, it should be delivered evenly. Attention must be paid to the degree of sparging, because excessive sparging washes away tannic acids from the filter layer, which is not desirable for technological and sensory purposes. The wort that runs off during lautering should be as clear as possible, so that no particles that could disintegrate further during wort boiling and only small amounts of the long-chain fatty acids, which destroy foam, can get into the kettle. (BRIGGS et al. 2004)

After lautering the wort is collected in the boiling kettle and boiled with the addition of hops. **Wort boiling** can roughly be divided in two processes: hot holding and evaporation. During hot holding, different chemical reactions take place such as hop alpha-acid isomerization, development of aroma substances, development of color and dissolution processes, as well as inactivation of enzymes and sterilization. During wort boiling the proteins and protein tannin complexes (hot break) need to be eliminated to obtain clarified wort. If the protein coagulation is too strong, foam-positive high molecular proteins are also precipitated that worsen the stability of the foam. Insufficient protein coagulation leads to colloidal unstable beers. For the customer turbid beer is a reason for reclamation in case of the most popular crystal-clear international lager beers. Turbidity-causing substances can, however, be partially removed by absorption before or during beer filtration using filtering aids.

The Maillard reactions that occur at about 80 °C generate new aroma substances. Of particular importance are Strecker aldehydes developed from amino acids that influence the taste stability of the beer. These are primary and secondary products of the Maillard reaction, and can be reduced depending on volatility, boiling system used and evaporation. Evaporation serves to remove undesired aroma substance such as myrcene from hops and different carbonyl as well as sulfur substances, especially dimethyl sulfide (DMS). Aroma substances from lipid metabolism are also reduced by evaporation during wort boiling. Moreover, the original extract is adjusted. This is necessary to ensure the product constancy and legal requirements/marketability of the beer.

At the end of the boil the wort should be absolutely clear but contain, suspended in it, the remains of hops and flocs of trub or hot break. The devices most often installed for hot wort clarification in modern breweries are **whirlpool** tanks. Hot wort is injected tangentially, the tangential injection causes the wort in the vessel to rotate. After filling the vessel wort rotation gradually slows. During rotation, the particles in the wort and the liquid are driven outwards by centrifugal force. The vertical pressure of the raised liquid at the edges tends to drive the liquid downwards and the particles strike the wall, move down with the liquid flow, and tend to settle to the bottom. Deposited particles spiral towards the center of the base. After 20-30 minutes the wort has cleared and the solids are deposited as a cone in the center of the base of the vessel, surrounded by a clear, annular space. Hot break contains roughly 50-60% crude protein, 20-30% tannin, 15-20% resins and 2-3% ash. (KROTTENTHALER et al. 2009)

After clarification, the hot **wort must be cooled** to the temperature at which it is **inoculated with yeast**. Traditionally this is about 15-22 °C for ales and 6-12 °C for lagers, but other temperatures are also used. The cooling should be carried out rapidly and under aseptic conditions to stop chemical reactions continuing and to minimize chances of growth of any contaminating microbes. As the wort cools it becomes hazy as a cold break is formed. This may or may not be removed. In addition, the wort must be charged with oxygen to an appropriate level. Avoidance of oxidation is key throughout the brewing process, especially at high temperature steps, however, at this point, oxygen is intentionally added to the cold hopped wort, which can be used by yeast cells to proliferate at the beginning of fermentation. (KROTTENTHALER et al. 2009) The second part of the brewing process is presented on Figure 6.

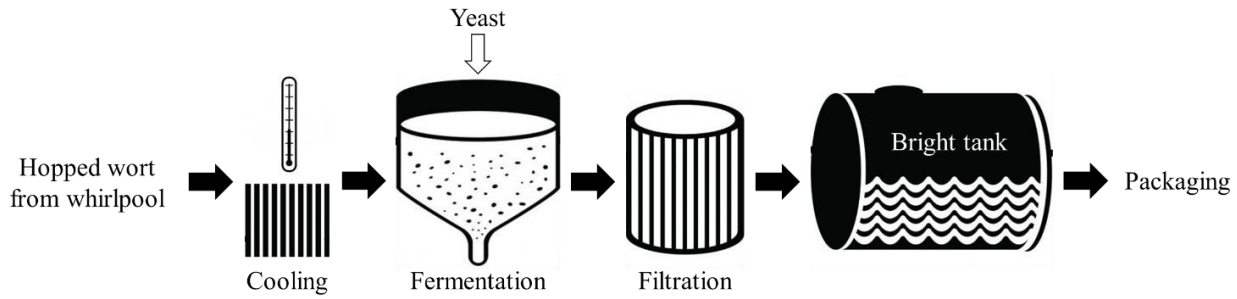


Figure 6: Brewing process part II.

Transferring wort into beer is the next main step. **Fermentation** is the step where there is the biggest difference between the input and output of the process during brewing. Fermentation means to metabolize substrates into products by the activity of microorganisms. In case of

brewing, yeast transfers sugars to ethanol and CO_2. During fermentation byproducts are formed, which have a considerable effect on the aroma profile and the taste of the resulting beer. Fermentation is started by adding yeast to the wort. Brewer's yeasts are mainly two types, called top-fermenting (*Saccharomyces cerevisiae*) and bottom-fermenting yeast (*Saccharomyces pastorianus*). The Saccharomyces yeasts are facultative anaerobes, which means that they can easily adjust their metabolism from aerobic to anaerobic conditions. Top fermentation is the oldest method of beer production and was the only one used until about the middle of the nineteenth century.

During the first phase of fermentation yeast doubles or triples its mass. For the build-up of cell substances yeast needs mostly amino acids, which it either takes from the fermenting substrate or must synthesize by itself. Apart from proteins, lipids have to be synthesized for yeast propagation because they are important components of the cell wall and are also needed for the uptake of nutrients. Molecular oxygen is necessary for the synthesis of these lipids from acetyl coenzyme A. Finally, yeast also requires minerals for the stabilization of its enzyme systems. After the main fermentation, when the appropriate alcohol content of the beer was formed, beer is lagered at temperatures of 0 to −2 °C to clarify and that the right amount of carbon dioxide is absorbed in the beer. During this process, its filterability and its colloidal stability are improved. Frequently, fermentation, maturation, and conditioning take place in the same vessel (uni- or one-tank process).

The metabolic activity of brewer's yeast is very complex, it is one of the most researched microorganisms, as a result of which its carbohydrate and nitrogen metabolism is well known, however, for example, its vitamin metabolism, including folic acid metabolism, is a less researched area. Due to the complexity of the topic, I did not place much emphasis on examining the changes that take place during fermentation, as this could be the topic of a separate dissertation.

Worldwide more than 90% of beers have brilliant clarity. During lagering, besides maturation the sedimentation of solid materials also takes place. The beers following maturation should not only have desirable, stable flavors but must also display stability with respect to haze, i.e., the beers must be bright and remain so during the period from dispatch from the brewery to drinking. Therefore, in addition to removing yeast, beers must have the precursor constituents of haze removed to ensure long-term stability. The purpose of **filtration** is to preserve the beer so that no visible changes occur in the long run and the beer keeps its original appearance. Generally, the filtration steps fulfil two roles:

- To remove suspended materials from the green beer (the real filtration).

- To unhinge potential turbidity formers (stabilization).

A range of substances can cause non-biological haze in beer, i.e., β-glucans, which can often lead to hazes not easily seen by eye, but which cause high levels of light scattering in 90° haze meters; α-glucans (starch), which can behave similarly to β-glucans; pentosans, which may be derived from wheat-based adjuncts; dead bacteria from malt; oxalate from calcium deficient worts. However, the most common, important and troublesome type of non-biological haze is that deriving from the cross-linking of proteins and polyphenols and it is the elimination of the precursors of these polymers to which **beer stabilization** treatments are directed. The most effective beer treatment with respect to haze stability is the cold storage of the beer for about seven days at -1 to -2 °C. This technique allows a reduction in the cost of other beer treatments designed to remove potential haze-forming proteins and polyphenols.

Colloidal haze in beer arises from the formation of protein-polyphenol complexes during beer storage. Fresh beer contains acidic proteins and numerous polyphenols. These can come together by loose hydrogen bonding, but the associations formed are too small to be seen by the naked eye. These polyphenols, called flavanoids, can further polymerize and oxidize to produce condensed polyphenols, which have been called tannoids. These tannoids can bridge by hydrogen bonding across a number of proteins to form a reversible chill haze. This haze forms at around 0 °C but redissolves when the beer is warmed to 15 °C. After further storage of the beer strong bonds can form between the tannoids and proteins and irreversible, permanent haze is formed. The rate at which this haze is formed, and its extent of formation depends on the raw materials used in wort preparation and the process conditions. This model suggests that effective stabilization should be achieved by removing from the beer the constituents of the haze, i.e., the tannin sensitive proteins

and/or the polyphenols.

For the removal of proteins silica gels are used because silica gels are excellent protein adsorbents. Silica gels can be added to beer during maturation, but their most attractive characteristic is their usefulness when dosed into the beer stream prior to filtration. Adsorption is therefore achieved with a very short contact time (80% of the adsorption is achieved in < 3 minutes) between the silica and the beer.

For the removal of polyphenols PVPP (polyvinylpolypyrrolidone) is used. This is a cross-linked polymer, which is insoluble in water, alcohol and acid and hence has a high surface area for adsorption of haze-forming polyphenols. This occurs at the surface of the material by strong hydrogen bonding. PVPP can be employed as a single use agent and in this situation the insoluble

PVPP-polyphenol complex is removed on the filter with kieselguhr depth filtration. The PVPP can be added to the beer stream along with kieselguhr at 0 °C and, like silica gel, a short contact time is effective, although in this case about ten minutes is needed from the point of contact with the beer to removal on the filter. PVPP can also be used as a regenerable product. Washing with hot caustic soda solution which breaks the PVPP-polyphenol bonds.

Finally, **the filtered beer is filled** into different packaging. In addition to marketing aspects, the properties of the packaging material, in conjunction with the product-specific idiosyncrasies of the beer concerned, have to be given due to consideration by the beer bottler or canner when choosing the appropriate container.

For filling beer, there are four main categories of packaging in use worldwide: glass bottles, cans made of aluminum or tinplate, plastic bottles made of polyethylene-terephthalate (PET) and polyethylene-naphthalate (PEN), and kegs.

The following factors are important for assessing whether and to what extent these packaging materials are suitable for packaging beer: light-proofing characteristics, barrier properties for preventing the escape of CO_2 or the entry of oxygen, and inertness in terms of mass transfer between the packaging material and the product, plus ability to withstand mechanical stresses and breakage. (BRIGGS et al. 2004)

3.7 Folate

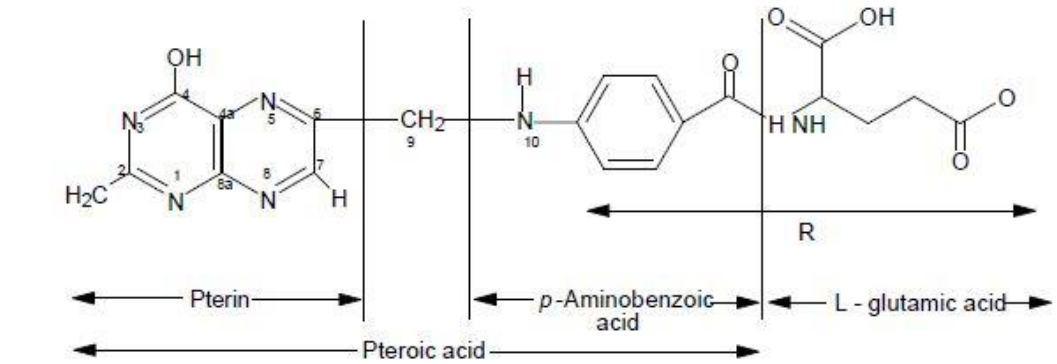
One of the health-promoting components I studied during the malting and brewing process is folate. First, the health-related effects, the metabolism, the natural occurrence, and quantification of folate are presented. Then I review current knowledge on the evolution of folate during malting and brewing.

Folate belongs to the B vitamin family. It acts as a coenzyme in single-carbon transfer reactions, principally as acceptors and donors of one-carbon units. Through this role, folate coenzymes mediate the metabolism of nucleic and amino acids, and thus fill an important function in purine and pyrimidine metabolism and the remethylation of homocysteine to methionine. Methionine is the immediate precursor of S-adenosyl methionine, which functions as the universal donor in many transmethylation reactions, including the methylation of DNA, histones and other proteins. Elevated concentration of total homocysteine in blood (hyperhomocysteinemia) is the risk factor for occlusive vascular disease (MAYER et al. 2001). Thus, folate deficiency can lead to severe metabolic and clinical consequences. (PITKIN 2007, MOLL et al. 2017) It also has an important role in prevention of neural tube defects in fetus. Insufficient folate intake is associated with

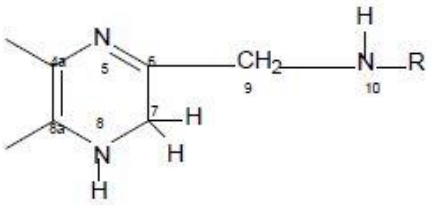
megaloblastic anemia, an increased risk of cardiovascular diseases, stroke, and certain types of cancer, and may be associated with dementia and Alzheimer's disease (ANDERSSON et al. 2008, HÜBNER et al. 2013). Another aspect of the increasing interest in folate in terms of health promotion is the discovery that it may play an important role as an antioxidant, both by preventing the adverse effect of reactive oxygen species (ROS), as well as by inhibiting lipid peroxidation (MEROLA et al. 2013).

Foods deliver folate mostly in bound form that is, combined with a string of amino acids (all glutamate), known as polyglutamate. During its **metabolism** enzymes on the intestinal cell surfaces hydrolyze the polyglutamate to monoglutamate, folate with only one glutamate attached, and several single glutamates. The monoglutamate is then attached to a methyl group (CH_3) and delivered to the liver and other body cells. To activate folate, the methyl group must be removed by an enzyme that requires the help of vitamin B12. Without that help, folate becomes trapped inside cells in its methyl form, unavailable to support DNA synthesis and cell growth. The liver incorporates excess folate into bile that is then sent to the gallbladder and gastrointestinal (GI) tract. Thus, folate travels in the same enterohepatic circulation as bile. This complicated system for handling folate is vulnerable to GI tract injuries. Because folate is actively secreted back into the GI tract with bile, it can be reabsorbed repeatedly. If the GI tract cells are damaged, then folate is lost. Such case is in alcohol abuse. Folate deficiency rapidly develops and ironically, further damages the GI tract. Folate is active in cell multiplication and the cells lining the GI tract are among the most rapidly replaced cells in the body. When unable to make new cells, the GI tract deteriorates and not only loses folate, but fails to absorb other nutrients as well. (WHITNEY et al. 2016)

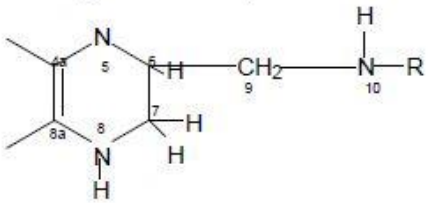
Folate occurs in natural foods as several vitamers (Figure 7), reduced derivatives of folic acid (pteroyl-L-glutamic acid (PGA)) which mainly exist as polyglutamates, folic acid, which is a monoglutamate, is the oxidized and most active form of the vitamin found rarely in food, it is the form used in vitamin preparations and food fortification. (ANDERSSON et al. 2008) The folates that occur in nature are the 7,8-dihydro- and 5,6,7,8-tetrahydro-reduced forms of PGA, resulting in dihydrofolate (DHF) and tetrahydrofolate (THF). Although only the monoglutamate form is metabolically active, approximately 80% of all native folates are polyglutamates, with 5–8 conjugated glutamate units most abundant. (VAN WYK et al. 2014)



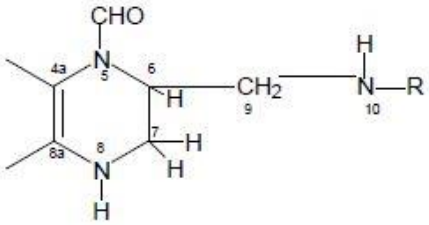
Pteroylmonoglutamic acid (PGA or PteGlu)



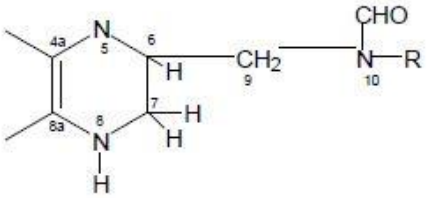
7,8-Dihydropteroylmonoglutamic acid (DHF)



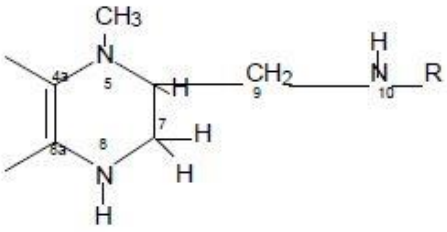
5,6,7,8-Tetrahydropteroylmonoglutamic acid (THF)



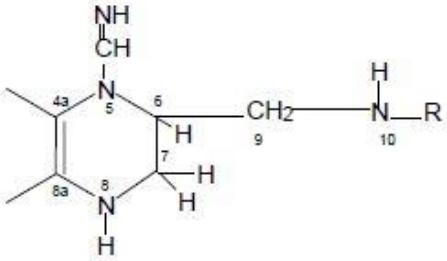
5-Formyl-THF (5-CHO-THF)



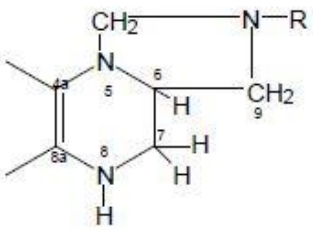
10-Formyl-THF (10-CHO-THF)



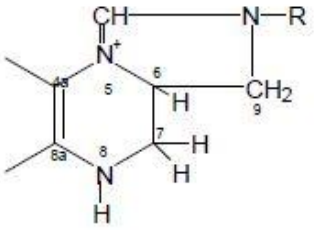
5-Methyl-THF (5-CH_3 -THF)



5-Formimino-THF (5-CH=NH-THF)



5,10-Methylene-THF (5,10-CH_3 -THF)



5,10-Methenyl-THF (5,10=CH-THF)

Figure 7: Folate vitamers (VAN WYK et al. 2014)

3.7.1 Folate quantification

The quantification of folates in food is complicated due to the numerous forms of native folates, their instability, the complexity of food matrices and the relatively low concentration of the analytes (VAN WYK et al. 2014). For their analysis, microbiological assay, and high-performance liquid chromatography (HPLC) with various detectors (e.g., UV, fluorescence, and mass spectrometry) are commonly applied. There are pros and cons of both methods. Whereas the microbiological assay is very sensitive but reveals only the total amount of folates, HPLC allows one to differentiate and quantitate the single vitamers. RINGLING et al. (2016) reported, in connection with the HPLC method, that insufficient deconjugation can lead to lower results and HPLC methods can be inaccurate when unequivocal UV extinction coefficients are used for calibration of the single vitamers. On the other hand, they have reported that the microbiological assay may give inaccurate results because of different responses and stabilities of the single vitamers, furthermore, results are only available after 2–5 days and shows a lower growth response when PGA is used as the calibrator, compared to 5-CH_3-THF, resulting in an underestimation of the folate concentration to some extent (PFEIFFER et al., 2010; RADER et al., 1998). (VAN WYK et al. 2014)

The microbiological assay used to be the only method given official status by the Association of Official Agricultural Chemists (AOAC) (IYER et al. 2009), but two new methods were awarded "AOAC First Action Official Method^{SM}" status. AOAC 2011.05 is entitled "An Optical Biosensor Assay for the Determination of Folate in Milk and Nutritional Dairy Products" and is based on a surface plasmon resonance (SPR) optical biosensor. AOAC 2011.06 "Total Folates in Various Foods by Trienzyme Extraction and UPLC-MS/MS Quantitation" uses UPLC-MS/MS to measure total folates of seven folate forms (SULLIVAN 2012, VAN WYK et al. 2014)

The principle of the turbidimetric **microbiological assay** is the specific growth-dependence of *Lactobacillus rhamnosus* on folate. The mostly applied microorganism, *Lactobacillus rhamnosus* (ATCC 7469) gives similar response to monoglutamates, diglutamates and triglutamates but a decreasing activity with further increase in chain length. Since folate mainly exists as polyglutamate in natural foods, a certain degree of deconjugation is necessary when cereal grains, malts or beer samples are examined. For this deconjugation mostly chicken or pork pancreatin is used because their wide range of enzyme activity (e.g., protease, α- and β-amylase) (RINGLING et al. 2017).

3.7.2 Evolution of folate during malting

Cereals are considered a good source of folate (Table 1). The folate content of beer originates from two main sources: grains and yeast. As more than 90% of commercial beers are filtered, grains are the primary source of folate in the majority of the final products. The most often used cereal for beer brewing is barley but wheat, oat and rye are also applied besides barley. Grains used for brewing are mainly used in their malted form. Among the steps of malting germination has been reported as a way to improve folate content in wheat (KOEHLER et al. 2007), barley (WALKER et al. 2002), oats (WILHELMSON et al. 2001) and rye (LIUKKONEN et al. 2003). During germination, folates are synthesized de novo, as they are needed for the cell growth and differentiation that is occurring. Folate is very important during seedling development due to the occurrence of major cellular events that involve one-carbon metabolism. It has been reported that the folate content of barley grains increases on average between two- and three-fold during malting, and most of the increase was observed during the first two days of germination. (JÄGERSTAD et al. 2005)

Table 1: Folate content of different cereals

Sample	Folate content [µg/100g dry matter]	Reference
10 barley genotypes	51.8 - 78.9	ANDERSSON et al. 2008
5 barley cultivars in three harvest years	56.3 - 77.3	EDELMANN et al. 2013
5 oat genotypes	49.5 - 60.4	SHEWRY et al. 2008
150 wheat genotypes	32.3 - 77.4	PIIRONEN et al. 2008
10 rye varieties	63.0 - 78.0	KARILUOTO 2008

WALKER (2003) conducted a comprehensive study of the folate content of cereals and malts and the factors influencing the folate content. They reported that the folate content of commercial barley malts is in the range of 200-400 µg/100g. This level is 3-4-fold higher than the levels of folate in unmalted cereals. The highest folate values were measured in high diastatic potential malts (average 400 µg/100g), and in the co-product of malting, roots which contained folate levels up to 10 times that of commercial malts. Also, a genetic influence was observed on this process in that some varieties produced more folate on malting than others. In the pilot maltings, small-scale work also suggested that the extent of germination was an influential factor on folate content. Darker roasted products and crystal malts had much lower folate values, with folate in the darkest products being undetectable. Roasting trials suggested that when the product reaches a temperature of about 200 °C, the folate in the cereal starts to degrade. The data of 60

barley malts showed that there was indeed a statistically significant varietal effect, with some varieties accumulating more folate than others. This result suggests that there must be a genetic component to the ability to accumulate folate.

3.7.3 Evolution of folate during brewing

There are several publications about the folate content of beers originating from different countries of the world but there is only one publication about the study of the folate content during some technological steps of brewing. PÓO-PRIETO et al. (2011) investigated the folate content of Spanish beers which ranged between 1.6 and 2.6 μg/100 ml, WALKER (2003) reported values for total folate in Bavarian wheat beers ranging from 4.7 to 12.5 μg/100 ml, PIETERCELIE et al. (2003) claimed that 14% of refermented Belgian beers contained more than 20 μg/100 ml, while BERTUZZI et al. (2020) reported the folate content of 80 beers (40 small-scale, 40 large-scale) available in Italy ranging between 1.55 and 10.48 μg/100 ml. Based on these results it is hard to draw conclusions about the general folate content of beer but the study of brewing technology can help to understand the differences.

The only publication about the study of the folate content during some technological steps of brewing, as mashing, fermentation and packaging in pilot and commercial scale breweries was published by JÄGERSTAD et al. (2005). But this article does not provide any information about the technological parameters (temperature, time, volume, facilities and so on), furthermore does not provide concrete concentrations of folate content, it reports the results in the dimension of folate content relative to barley. In order to draw concrete conclusions, it is necessary to know the technological parameters and facilities accurately.

3.8 Antioxidants

In addition to folates, antioxidant compounds are the other group of health-promoting components that I study in my dissertation. It has been a hot topic in the last twenty years and has been studied widely, even during certain steps of brewing and malting. A comprehensive research, that includes raw materials and technologies have not been performed so far, which could add to and make the existing knowledge even more valuable.

Antioxidants are compounds which can help us to retain our health. **The main role of antioxidants** in human health is to attenuate oxidative stress. Oxidative stress arises from overproduction of reactive oxygen or nitrogen species (ROS/RNS) (SHAHIDI et al. 2012). **ROS** is a collective term used for a group of oxidants, which are either free radicals or molecular

species capable of generating free radicals. These free radicals are produced under normal physiological and pathological conditions in our organism and play an important role in pathological processes and regulatory activities. Intracellular generation of ROS mainly comprises superoxide ($O2^{\bullet-}$) radicals and nitric oxide ($NO^{\bullet}$) radicals. Under normal physiological conditions, nearly 2% of the oxygen consumed by the body is converted into $O2^{\bullet-}$. ROS percentage increases during infections, exercise, exposure to pollutants, UV light, ionizing radiation, etc. $NO^{\bullet}$ is an endothelial relaxing factor and neurotransmitter, produced through nitric oxide synthase enzymes. $NO^{\bullet}$ and $O2^{\bullet-}$ radicals are converted to powerful oxidizing radicals like hydroxyl radical ($^{\bullet}OH$), alkoxy radicals ($RO^{\bullet}$), peroxyl radicals ($ROO^{\bullet}$), singlet oxygen ($^1O2$) by complex transformation reactions. Some of the radical species are converted to molecular oxidants like hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl). Sometimes these molecular species act as source of ROS. Typically, low concentration of ROS is essential for normal physiological functions like gene expression, cellular growth and defense against infection. Sometimes they also act as the stimulating agents for biochemical processes within the cell. Depending upon their nature, ROS (e.g. $^{\bullet}OH$ radicals) react with biomolecules such as lipid, protein, and DNA, produce different types of secondary radicals like lipid radicals, sugar and base derived radicals, amino acid radicals and thiyl radicals. These radicals in presence of oxygen are converted in peroxyl radicals. Peroxyl radicals are critical in biosystems, as they often induce chain reactions1.

Uncontrolled generation of ROS can lead to their accumulation causing oxidative stress in the cells. Therefore, cells have evolved defense mechanisms for protection against ROS mediated oxidative damage. These include antioxidant defenses to keep a check on the generation of ROS. An **antioxidant** is a substance that is present at low concentrations and significantly delays or prevents oxidation of the oxidizable substrate. Antioxidants are effective because they can donate their own electrons to ROS (Figure 8) and thereby neutralizing the adverse effects of the latter. In general, an antioxidant in the body may work at three different levels: prevention - keeping formation of reactive species to a minimum e.g. desferrioxamine, interception - scavenging reactive species either by using catalytic and non-catalytic molecules e.g. ascorbic acid, alpha-tocopherol, and repair - repairing damaged target molecules e.g. glutathione. The antioxidant systems are classified into two major groups, enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants present in the body include superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) that act as body's first line of defense against ROS by catalyzing their conversion to less reactive or inert species. Several low molecular weight molecules present inside the cell provide secondary defense against free

radicals. A few examples of such molecules include glutathione (GSH), α-tocopherol, ascorbate, bilirubin, etc. These agents either scavenge the ROS directly or prevent the production of ROS through sequestration of redox active metals like iron and copper. (KUNWAR et al. 2011)

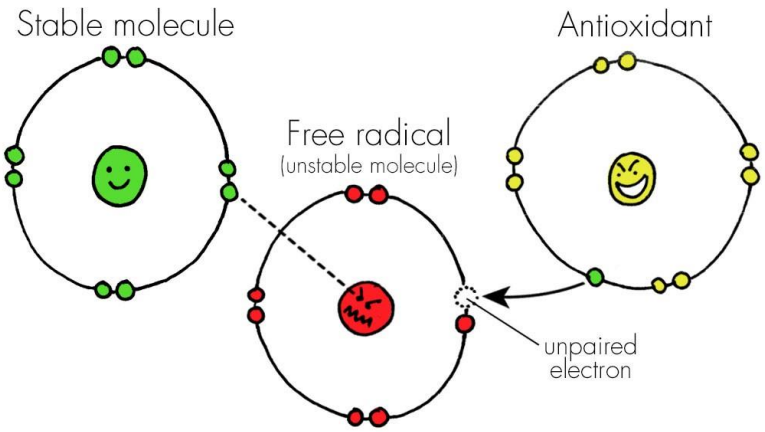


Figure 8: Electron donation of antioxidant molecules

Although cells are equipped with an impressive repertoire of antioxidant enzymes as well as small antioxidant molecules, these agents may not be sufficient to normalize the redox status during oxidative stress. Under such conditions supplementation with exogenous antioxidants is required to restore the redox homeostasis in cells. Since several plant products are rich in antioxidants and micronutrients, it is likely that dietary antioxidant supplementation protects against the oxidative stress mediated disease development. Therefore, to maintain optimal body function, antioxidant supplementation has become an increasingly popular practice.

The primary concern regarding antioxidant supplementation is their potentially deleterious effects on ROS production (pro-oxidant action). In fact, some negative effects of antioxidants when used in dietary supplements (flavonoids, carotenoids, vitamin C and synthetic compounds) have emerged in the last few decades. Mechanistic investigation has revealed that antioxidants may exhibit pro-oxidant activity depending on the specific set of conditions. Of particular importance are their dosage, redox conditions and also the presence of free transition metals in cellular milieu. (KUNWAR et al. 2011)

Antioxidants have specific role in several conditions e.g.: burns, ageing, cancer, cardiovascular diseases, reperfusion injury, hypertension, atherosclerosis, diabetes, cataract, inflammatory diseases, rheumatoid arthritis, AIDS, diseases of the central nervous system (Parkinson's disease, Huntington's disease, Alzheimer's disease, Schizophrenia) and infertility. (NEERAJ et al. 2013)

3.8.1 Polyphenols

Polyphenols are the most researched compounds among antioxidants. Due to their structural diversity and their varied role in the human body, their analytical quantification and qualification and mapping of their bioactive functions present many challenges. Polyphenols with several thousand structural variants are secondary metabolites of plants and represent a huge gamut of substances having aromatic ring(s) bearing one or more hydroxyl moieties. These molecules in plants are generally involved in defense against ultraviolet radiation or aggression by pathogens.

Interest in the health effects of polyphenols has exponentially increased in recent years. From the nutritional point of view, polyphenols have been widely reported to have beneficial effects on human health in many areas (WATSON et al. 2018). Polyphenols are the most abundant antioxidants in the diet. Their total dietary intake could be as high as 1 g/d, which is much higher than that of all other classes of phytochemicals and known dietary antioxidants.

One of the major difficulties of elucidating the **health effects of polyphenols** is the large number of phenolic compounds found in food, yielding differing biological activities. They can exhibit their effects through different mechanisms, including functioning as free radical scavengers, reducing agents, singlet oxygen quenchers and chelators of divalent transition metals such as iron and copper. Polyphenols can release electrons to free radical species, generating phenoxy radicals that are relatively stable (VANDERHAEGEN et al. 2006). Current evidence strongly supports a contribution of polyphenols to the prevention of cardiovascular diseases, cancers, and osteoporosis and suggests a role in the prevention of neurodegenerative diseases and diabetes mellitus. However, our knowledge still appears too limited for formulation of recommendations for the general population or for particular populations at risk of specific diseases. Much of the evidence on the prevention of diseases by polyphenols is derived from in vitro or animal experiments, which are often performed with doses much higher than those to which humans are exposed through the diet. The active compounds may not be the native polyphenols found in food, which are most often tested in in vitro studies, they are more likely to be metabolites. Polyphenols are extensively conjugated in the body, and nonconjugated metabolites most often account for a minor fraction of the circulating metabolites. (SCALBERT 2005)

Polyphenols clearly improve the status of different oxidative stress biomarkers. Much uncertainty persists, however, regarding both the relevance of these biomarkers as predictors of disease risk and the appropriateness of the different methods used. Significant progress has been made in the field of cardiovascular diseases, and today it is well established that some

polyphenols, administered as supplements or with food, do improve health status, as indicated by several biomarkers closely associated with cardiovascular risk.

A considerable body of literature supports a role for **oxidative stress** in the pathogenesis of age-related human diseases and a contribution of dietary polyphenols to their prevention. The complex relationships between antioxidant status and disease are still poorly understood and have been studied intensively. For many years, polyphenols and other antioxidants were thought to protect cell constituents against oxidative damage through scavenging of free radicals. However, this concept now appears to be an oversimplified view of their mode of action. More likely, cells respond to polyphenols mainly through direct interactions with receptors or enzymes involved in signal transduction, which may result in modification of the redox status of the cell and may trigger a series of redox-dependent reactions. Both antioxidant and prooxidant effects of polyphenols have been described, with contrasting effects on cell physiologic processes. As antioxidants, polyphenols may improve cell survival, as prooxidants, they may induce apoptosis and prevent tumor growth. However, the biological effects of polyphenols may extend well beyond the modulation of oxidative stress. One of the best-known examples involves the interaction of soy isoflavones with estrogen receptors and the effects of these compounds on endocrine function. These effects could explain the prevention by isoflavones of bone resorption among postmenopausal women. (SCALBERT 2005)

Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. Distinctions are thus made between the phenolic acids, flavonoids, stilbenes, and lignans. The flavonoids, which share a common structure consisting of 2 aromatic rings (A and B) that are bound together by 3 carbon atoms that form an oxygenated heterocycle (ring C), may themselves be divided into 6 subclasses as a function of the type of heterocycle involved: flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (catechins and proanthocyanidins). In addition to this diversity, polyphenols may be associated with various carbohydrates and organic acids and with one another. (KUNWAR et al. 2011, MANACH 2004)

Phenolic acids

Two classes of phenolic acids can be distinguished: derivatives of benzoic acid and derivatives of cinnamic acid (Figure 9). The hydroxybenzoic acid (protocatechuic acid, gallic acid) content of edible plants is generally very low, while the hydroxycinnamic acids are more common which consist chiefly of p-coumaric, caffeic, ferulic and sinapic acids. These acids are rarely found in

the free form, except in processed food that has undergone freezing, sterilization or fermentation. The bound forms are glycosylated derivatives or esters of quinic acid, shikimic acid and tartaric acid. Ferulic acid is the most abundant phenolic acid found in cereal grains, which constitute its main dietary source. The ferulic acid content of wheat grain is 0.8–2 g/kg dry weight, which may represent up to 90% of total polyphenols. Ferulic acid is found chiefly in the outer parts of the grain. The aleurone layer and the pericarp of wheat grain contain 98% of the total ferulic acid. Ferulic acid is found mainly in the trans form, which is esterified to arabinoxylans and hemicelluloses in the aleurone and pericarp. Only 10% of ferulic acid is found in soluble free form in wheat bran. Several dimers of ferulic acid are also found in cereals and form bridge structures between chains of hemicellulose. (MANACH 2004)

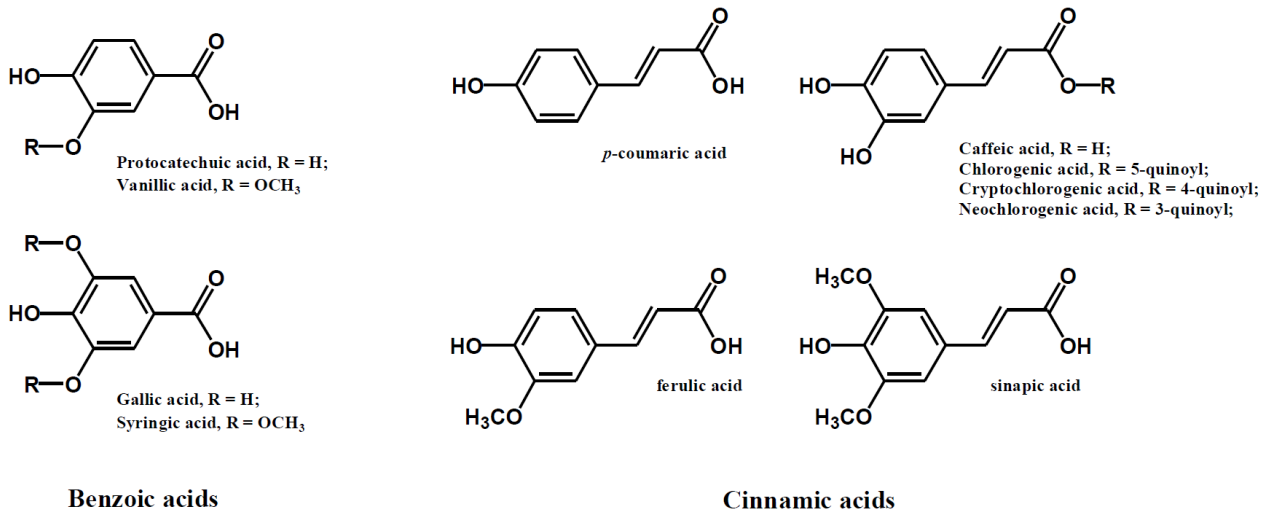


Figure 9: Phenolic acids (TSAO 2010)

Flavonoids

Flavones, flavonols, flavanones, flavanonols flavonoid subgroups are the most common, and almost ubiquitous, throughout the plant kingdom (Figure 10).

Flavonols are the most ubiquitous flavonoids in foods, and the main representatives are quercetin and kaempferol. These compounds are present in glycosylated forms. The associated sugar moiety is very often glucose or rhamnose, but other sugars may also be involved. These flavonols accumulate in the outer and aerial tissues (skin and leaves) because their biosynthesis is stimulated by light.

Flavones are much less common than flavonols in fruit and vegetables. Flavones consist chiefly of glycosides of luteolin and apigenin. Cereals such as millet and wheat contain C-glycosides of flavones.

In human foods, flavanones are found in tomatoes and certain aromatic plants such as mint, but they are present in high concentrations only in citrus fruit. The main aglycones are naringenin in grapefruit, hesperetin in oranges and eriodictyol in lemons. They are generally glycosylated by a disaccharide.

Isoflavones are flavonoids with structural similarities to estrogens. Although they are not steroids, they have hydroxyl groups in positions 7 and 4' in a configuration analogous to that of the hydroxyls in the estradiol molecule. This confers pseudohormonal properties on them, including the ability to bind to estrogen receptors, and they are consequently classified as phytoestrogens. Isoflavones are found almost exclusively in leguminous plants. (MANACH 2004)

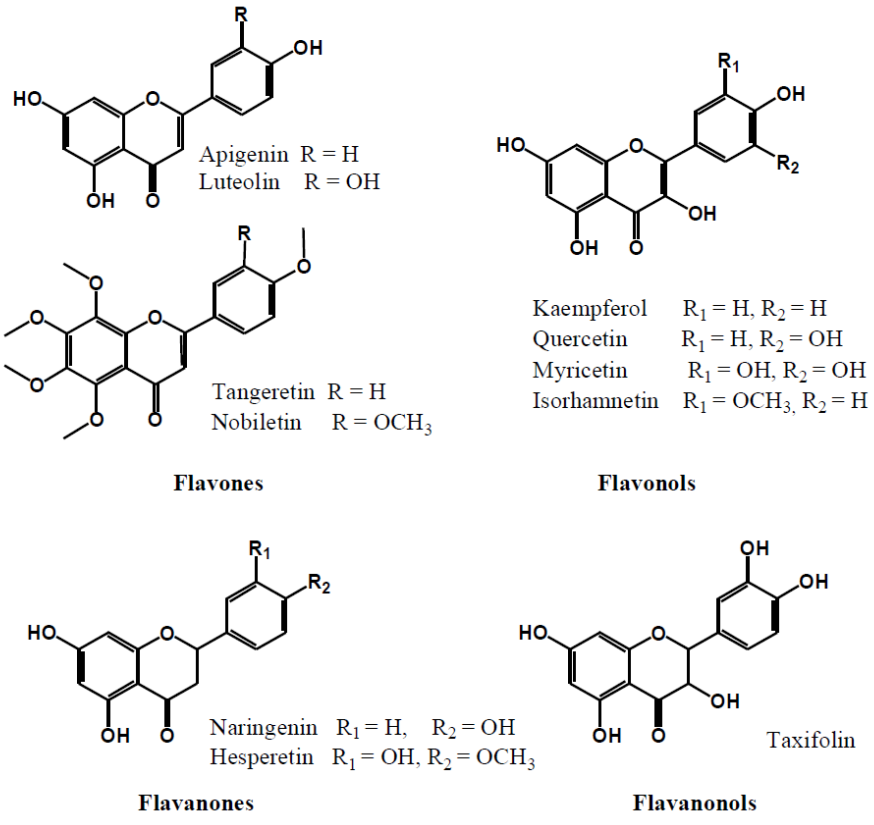


Figure 10: Flavones, flavonols, flavanones, flavanonols (Tsao 2010)

Flavanols exist in both the monomer form (catechins) and the polymer form (proanthocyanidins) (Figure 11). Catechin and epicatechin are the main flavanols in fruit, whereas gallocatechin, epigallocatechin and epigallocatechin gallate are found in certain seeds of leguminous plants, in

grapes and more importantly in tea. In contrast to other classes of flavonoids, flavanols are not glycosylated in foods.

Proanthocyanidins, which are also known as condensed tannins, are dimers, oligomers and polymers of catechins that are bound together by links between C4 and C8 or C6. Through the formation of complexes with salivary proteins, condensed tannins are responsible for the astringent character of fruit (grapes, peaches, kakis, apples, pears, berries, etc.) and beverages (wine, cider, tea, beer, etc.) and for the bitterness of chocolate. It is difficult to estimate the proanthocyanidin content of foods because proanthocyanidins have a wide range of structures and molecular weights.

Anthocyanins are pigments dissolved in the vacuolar sap of the epidermal tissues of flowers and fruit, to which they impart a pink, red, blue, or purple color. They exist in different chemical forms, both colored and uncolored, according to pH. (MANACH 2004)

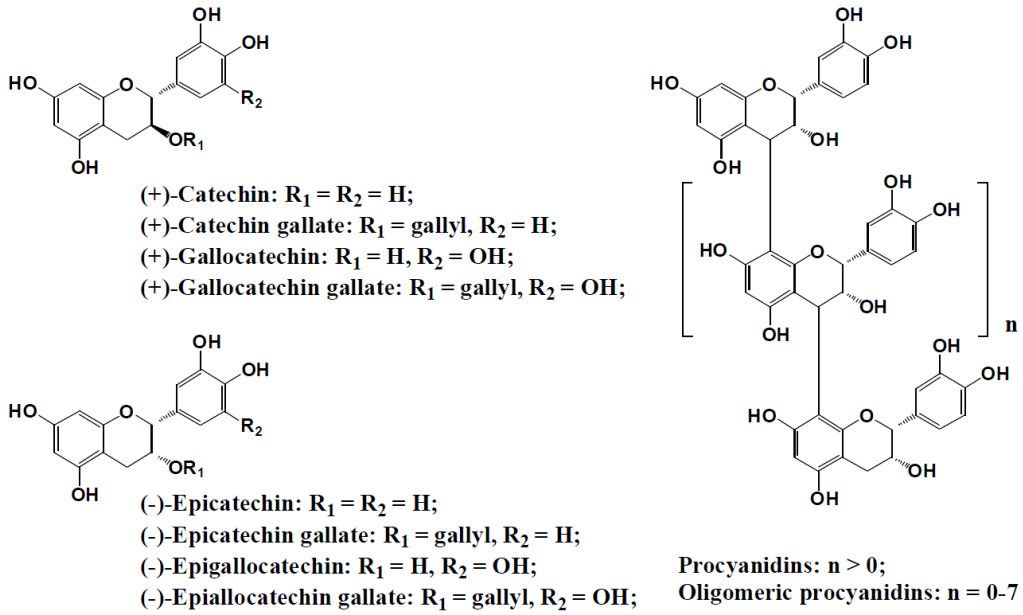


Figure 11: Flavanols

3.8.2 Role of phenolic compounds in brewing

Polyphenols influence, directly or indirectly, beer production because of their chemical properties and, in particular, because of their multiplicative reactivity. They can form many different compounds. Monomer and oligomer polyphenols can interact both in the aerobic and in anaerobic phase during the brewing process. The interactions during brewing between phenolics and proteins are certainly important. Flavanol derivatives are very susceptible to free-radical reactions initiated either by oxygen or plant oxidase/peroxidase enzymes. These reactions can

lead to cross linking, an increase in molecular size and the formation of polymeric flavanols which are strongly tanning (that is they bind to proteins and may precipitate them). The associations may be reversible, as in chill hazes, or may be irreversible, as in permanent hazes. During the reaction with proteins, they form a non-biological haze which will limit the shelf-life of beers. On the other hand, nowadays, turbidity is a required organoleptic property for some modern types of beers, e.g., New England IPA-s (India Pale Ale). The phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids) and flavonols readily dissolve during mashing and wort boiling and most survive into beer, but some ferulic acid is decarboxylated during boiling to yield 4-vinyl guaiacol a substance which in most beers confers an undesirable flavor.

Some phenols have antioxidant effects, that is, they block the oxidative reactions of other substances, at least sometimes by destroying free radical intermediates, but significance of phenol's antioxidant properties in brewing is unclear.

Phenolics are also credited with altering the astringency, the mouthfeel, and the aftertaste of beers. However, reports on the significance of involvement of phenolic compounds in beer flavor conflict. (BRIGGS et al. 2004)

GRESSER (2009) carried out an experiment with trial brews containing different concentration and composition of phenolic compounds. It was observed that color and foam stability are independent of the addition of polyphenols, but the polyphenols exhibited a negative effect on the colloidal stability in higher concentration, the susceptibility of haze increased with prolonged boiling. The reduction capacity proved to be higher due to the addition of the polyphenol fraction. Sensory evaluation was also carried out, the results were significant with regard to differentiation and preference. The beers with polyphenols contained a surprising balance in taste, and a very fine hoppy and fruity aroma, having an improved flavor stability. The presence of polyphenols did not impart a harsh bitterness if the duration of boiling is not excessive.

It is difficult to draw clear conclusions about the ideal polyphenol composition and concentration of beers. These compounds can contribute both positively and negatively to the quality of the final product, but it also depends on the type of beer to be produced.

3.8.3 Determination of antioxidant activity

The biggest problem in determining antioxidant activity is the lack of a validated assay that can reliably measure the antioxidant capacity of foods and biological samples. Several reviews have been published, and the opinions vary considerably. There seems to be no consensus of opinions, most probably due to the fact that the area of antioxidants is such a complex topic.

According to HUANG et al. (2005) there are two categories of assays which are applicable to determine antioxidant activity, these categories are hydrogen atom transfer reaction-based assays (HAT) and single electron transfer reaction-based assays (ET).

Most **HAT-based assays** monitor competitive reaction kinetics, and the quantitation is derived from the kinetic curves. HAT-based methods generally are composed of a synthetic free radical generator, an oxidizable molecular probe, and an antioxidant. The most popular HAT-based methods are the Oxygen radical absorbance capacity (ORAC), Total radical trapping antioxidant parameter (TRAP), Crocin bleaching assay, Inhibited oxygen uptake (IOU), Inhibition of linoleic acid oxidation and inhibition of LDL oxidation.

Electron transfer reaction-based assays involve two components in the reaction mixture, antioxidants, and oxidant. They are based on a reaction in which the probe (oxidant) is reduced by the antioxidant that causes color change which can be measured by a spectrophotometer. The degree of the color change is proportional to the antioxidant concentration. The reducing capacity of the antioxidant is usually expressed as ascorbic acid equivalent (AAE), trolox equivalent (TE) or gallic acid equivalent (GAE). These assays have the limitation that they are not selective to certain compounds, only suitable for determining the reducing capacity of the sample. On the other hand, significant correlation can be found between antioxidant activity and certain groups of antioxidants, e.g., polyphenol content in beer (Zhao et al. 2010). The most popular ET-based assays are Trolox equivalent antioxidant capacity (TEAC) also called 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity, Ferric ion reducing antioxidant parameter/power (FRAP), Total polyphenol content (TPC) by Folin-Ciocalteu reagent (FCR), Cupric reducing antioxidant capacity (CUPRAC) and diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. (HUANG et al. 2005)

During my research I applied ABTS radical scavenging activity, Ferric ion reducing antioxidant power, Total polyphenol content by Folin-Ciocalteu reagent, Cupric reducing antioxidant capacity and DPPH radical scavenging activity assays so I would present these in more detail below.

All the ET-based assays are suitable for determining a sample's reducing capacity, even the one called "total phenolic assay" or "total polyphenol content" using Folin-Ciocalteu reagent. Numerous publications applied TPC and an ET-based antioxidant capacity assay (e.g., FRAP, TEAC, etc.) and often found excellent linear correlations between the "total phenolic profiles" and "the antioxidant activity". This is not surprising if one considers the similarity of chemistry between these assays. The FC reagent is nonspecific to phenolic compounds as it can be reduced by many nonphenolic compounds (e.g. vitamin C, Cu(I), etc.). Phenolic compounds react with

FCR only under basic conditions. Dissociation of a phenolic proton leads to a phenolate anion, which can reduce FCR. This supports the notion that the reaction occurs through electron transfer mechanism. (HUANG et al. 2005)

ET-based assays use different oxidants that react with antioxidants in different ways and under different conditions. To ABTS radical scavenging activity metmyoglobin is used to generate $^{\bullet}OH$ in the presence of H_2O_2 which then reacts with ABTS to generate the radical cation. In the FRAP assay a ferric salt, Fe(III)(2,4,6-tripyridyl-s-triazine(TPTZ))$2Cl_3$, is used as an oxidant. The redox potential of Fe(III) salt (~0.70 V) is comparable to that of $ABTS^{\bullet-}$ (0.68 V). The CUPRAC method is based on reduction of Cu(II) to Cu(I). A chromogenic reagent, neocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline), forms a 2:1 complex with Cu(I), which has a maximum absorbance at 490 nm. DPPH is one of the few stable and commercially available organic nitrogen radicals and has a UV-Vis absorption maximum at 515 nm. The DPPH assay is technically simple, but some disadvantages limit its applications. Besides the mechanistic difference from the HAT reaction that normally occurs between antioxidants and peroxyl radicals, DPPH is a long-lived nitrogen radical, which bears no similarity to the highly reactive and transient peroxyl radicals involved in lipid peroxidation. Many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH. (HUANG et al. 2005)

In case of ET based assays first in general an oxidant is formed in the solution to which the sample is added. On the other hand, regarding ABTS radical scavenging activity antioxidants are already present in the solution which inhibit the generation of the free radical resulting in color change which can be measured at λ=405nm. ABTS radical scavenging activity and CUPRAC assays are carried out at physiological pH, furthermore in case of ABTS physiological salt and glucose solution is used to represent the conditions in the human body. Regarding TPC the pH is adjusted by a sodium carbonate solution to pH ~10 and FRAP assay is carried out under acidic conditions (pH 3.6).

With respect to FRAP assay the reaction is nonspecific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe3+ to Fe2+) ion formation. The change in absorbance is therefore, directly related to the combined or “total” reducing power of the electron donating antioxidants present in the reaction mixture. (GUPTA 2015)

CUPRAC assay is applicable to both hydrophilic and lipophilic antioxidants (unlike TPC and DPPH assays), has a selective action on antioxidant compounds without affecting sugars and citric acid commonly present in foodstuffs and has a capacity to assay –SH bearing antioxidants,

unlike FRAP. The CUPRAC assay describes the development of a simple and widely applicable antioxidant capacity assay for flavonoids, phenolic acids, hydroxycinnamic acids, thiols, synthetic antioxidants and vitamin C and E. (GUPTA 2015)

3.8.4 Quantification and qualification of polyphenols

Even though polyphenols share the common phenolic feature, due to the structural diversity, these phytochemicals vary significantly in their physicochemical properties. Owing to the chemical complexity and the frequent occurrence of polyphenols in plants, extraction, separation, identification, and analysis of polyphenols remain as challenging as ever, despite the recent advances in new instrumentation. The challenge is multiplied when the complex glycosylation and polymerization patterns and the various food matrices are considered.

To avoid degradation of native polyphenols, samples are often dried, frozen, or lyophilized before **extraction** because high moisture or water content aids enzyme activities. Heating and exposure to light and oxygen may affect the polyphenolic composition in many cases. For the majority of plant originated food samples, solvent extractions such as liquid/liquid partitioning and solid/liquid extraction are most frequently employed. The phenolic nature of polyphenols makes them relatively hydrophilic, thus free polyphenols, including aglycones, glycosides and oligomers, are extracted using water, polar organic solvents such as methanol, ethanol, acetonitrile and acetone, or their mixture of water. The pH of the extraction solvent is also important. For polyphenols, most extractions are carried out under acidic conditions because they are generally more stable in low pH, and the acidic condition helps polyphenols to stay neutral, thus readily extracted into organic solvents. This is done using weak acid or low concentrations of a strong acid. High acid concentration can cause hydrolysis of glycosides or acylglycosides and thus may give different pictures of native polyphenol profiles. On the other hand, not all polyphenols exist in the free form. Phenolic acids such as ferulic acid and lignans in grains are often bound to structural materials. Hydrolysis using acid or alkaline releases these phenolics which are partitioned into ethyl acetate or n-butanol.

The most widely used analytical tool for **quantification** of polyphenols is reversed-phase high performance liquid chromatography coupled with a diode array detector (RP-HPLC-DAD) and/or mass spectrometric detector (MS). The development of ultra high performance liquid chromatography (U-HPLC or UPLC) has significantly enhanced the performance of separation. Polyphenols can be separated with significantly enhanced efficiency and drastically reduced analytical time (to <1/10 of the time of a conventional HPLC). To obtain the identity of a polyphenol, the most common method is to compare the retention time of a particular compound

with the standard. The DAD can collect UV/visible (UV/Vis) spectral data as the compounds are separated, thus when a peak matches the retention time and the UV/Vis spectrum of a standard, it can be tentatively identified. On-line Liquid Chromatography coupled with Mass Spectrometer (LC-MS), particularly techniques such as tandem mass spectrometry that employs collision-induced dissociation (CID), can provide sufficient information for the final confirmation of most known polyphenols found in foods. In the absence of standards, compounds can also be identified on the basis of retention time and UV/Vis spectra found in the literature and quantified as the equivalent of a known polyphenol. (TSAO 2010)

3.8.5 Antioxidant potential of barley and malt

Malted barley is used in the second highest proportion after water in brewing. According to NARZISS (1976) malt contributes to 70-80% of the antioxidant activity of beer being the most important ingredient from this point of view. The most significant antioxidants in barley are polyphenols. The phenolic content of barley is influenced by biotic and abiotic factors, which affect plant physiology and secondary metabolites (MIKKELSEN et al. 2015). Phenolic compounds are predominantly found in the outer layers of the grain (husk, pericarp, testa, aleurone cells) bound to cell wall polysaccharides (KAHKONEN et al. 1999, NACZK 2004). During malting, the extractability of these compounds is increasing mainly due to enzymatic processes and better friability.

The other group of potential antioxidant compounds is developed during the heat treatment. In case of special malts (e.g., caramel, coloring) which are treated at high temperature (150–230 °C) at the end of the malting process, Maillard reaction and pyrolysis takes place intensively resulting in products that do have reducing ability, thus antioxidant capacity as well. (CARVALHO et al. 2015)

There are studies investigating the antioxidant potential of barleys and malts produced from them. ŠIBALIĆ et al. (2020) found that the most abundant phenolic compound found in the extracts of malts was epicatechin (16.88–20.75 mg/100 g). Other phenolic compounds were detected in lower concentrations: catechin (2.6–2.8 mg/100 g), syringic acid (0.87–0.90 mg/100 g), epicatechin gallate (0.30–0.51 mg/100 g), caffeic and p-coumaric acids (0.17–0.20 mg/100 g), ferulic and vanillic acids (0.06–0.10 mg/100 g), and p-hydroxybenzoic acid up to 0.07 mg/100 g. They reported that antioxidant activity of malt is in a high correlation with concentration of total polyphenols ($R = 0.9893$) and total flavonoids ($R = 0.8723$).

LU et al. (2007) showed that malting had significant influences on individual and total phenolic contents as well as antioxidant activities of barley. It was found that the most phenolic compounds identified in barley are (+)-catechin and ferulic acid, which both changed significantly during malting. The contents of some phenolic compounds and the antioxidant activities decreased significantly during steeping and the early stages of germination and then increased remarkably during the later stages of germination and subsequent kilning.

Different fractions of ten barley cultivars and their corresponding malts were investigated by DVOŘÁKOVÁ et al. (2008a). Phenolics extracted belonged to free phenols, soluble esters, and insoluble-bound fractions. TPC of the free fraction ranged from 37.7 to 167.2 mg GAE/kg of dried material (kgdw) for barley and between 34.1 and 72.3 mg GAE/kgdw for malt. The bound phenolic content ranged from 210.3 to 320.5 and between 81.1 and 234.9 mg GAE/kgdw for barley and malt, respectively. The contribution of bound phenolics to the TPC was significantly higher than that of free and esterified fractions. A decreasing tendency of TPC values is reported during the malting process. Catechin and ferulic acid were the most abundant phenolics in the free and bound fractions, respectively in agreement with the results of LU et al. (2007). The bound phenolics demonstrated a significantly higher antioxidant capacity compared to the free and esterified phenolics. During the malting process, a significant decrease of the bound phenolics was reported with a corresponding increase of the esterified fraction.

QUAN et al. (2018) observed that steeping and malting resulted in significant decreases in free phenolic compounds compared to raw grains. Contradictive results were obtained by CARVALHO et al. (2015) who found that malt samples were characterized by higher phenolic content comparing to corresponding barley varieties, revealing a significant increase of the levels of (+)-catechin and (−)-epicatechin during the malting process. LU et al. (2007) found that the contents of some phenolic compounds and antioxidant activities decreased significantly only during steeping and the early stages of germination but then increased remarkably during the later stages of germination and kilning. Furthermore, PEJIN et al. (2009) investigated individual phenolic acids during the malting process and observed significantly higher amounts of phenolic acids in malts than in corresponding barleys. CARVALHO et al (2015) also observed that industrial malting is responsible for modification on the phenolic profile from barley to malt, namely on the synthesis or release of sinapinic acid and epicatechin. Accordingly, the selection of the malting parameters, as well as the barley variety plays an important role when considering the quality and antioxidant stability of beer.

According to LEITAO et al. (2012) prodelphinidin B3 and procyanidin B3 are the two major contributors to the antioxidant activity of barley, in addition to catechin. They observed that

malting had a dramatic impact on these three compounds by resulting in a sharp decrease in their detected amounts and the associated antioxidant activities. Two other antioxidants, ferulic and sinapic acids, showed a better ability to withstand not only malting but also brewing steps. This agrees with the results of CAI et al. (2015) who identified ferulic acid (FA) and p-coumaric acid (p-CA) as two main phenolic acids, showing wide variations among 68 barley genotypes. The mean content of FA and p-CA were 2.15 μg/g and 1.10 μg/g in grains and 4.07 μg/g and 1.44 μg/g in malt, respectively. After malting, FA and p-CA content increased significantly in 55 and 37 genotypes and reduced in 2 and 14 genotypes, respectively.

It was found by ZHOU et al. (2020) that antioxidant activities, including TPC, DPPH, ABTS, and superoxide anion radical scavenging activities, reducing power and iron (II) chelating activity, were significantly impacted by the barley genotype, growth environment, and their interactions. The antioxidant activity was also influenced by malting conditions and were generally higher in the malted barleys of the thin kernel size fraction, four-day germination, and in samples processed by kilning, when compared to the plumper kernel size fractions, one and two days of germination, and freeze-dried samples, respectively.

It can be concluded that there is no consensus in the literature regarding to the antioxidant activity, phenolic content and composition, and trends during malting. Some studies report a continuous decrease in some components throughout the malting process, while some report a continuous increase, but there are also cases where some technological steps are attributed to an increasing effect while others are attributed to a reducing effect. This is a complex topic because the environmental properties, biotic and abiotic factors already present during barley cultivation affect the whole process, as a result of which some barley cultivars react differently to the impacts during malting, thus making it impossible to draw general conclusions on this topic.

3.8.6 Antioxidant potential of hops

Hop, the other important ingredient of beer not only regarding to antioxidants. It contains higher concentrations of polyphenols than barley malt (barley malt 50–100 mg/100 g, hops up to 4 g/100 g), but contributes to only 20-30% of total polyphenols in traditional beers (NARZISS 1976, ALMAGUER et al. 2014;). Hop cones contain 3% to 6% polyphenolic compounds related to dry weight (MOIR 2000). The majority of polyphenols are located in the strig and bract, with the exception of the prenylflavonoids, which are secreted from lupulin glands (ALMAGUER et al. 2014). According to BIENDL (2009) hop polyphenols can be split into 4 groups: flavonols, flavan-3-ols, phenolic carboxylic acids (derivatives of benzoic acid and cinnamic acid), and other phenolic compounds (prenylflavonoids, stilbenoids and so on).

There are studies investigating the antioxidant potential of hop varieties and their effect on wort and beer stability. KOWALCZYK et al. (2013) made experiments regarding to the extraction of antioxidants from hops. It was found that the total phenolic and total flavonoid contents (TFC), as well as antioxidant activity (ABTS scavenging activity, reducing power and metal chelating activity) of the hydroalcoholic extracts of hops were significantly higher than those of aqueous extracts. Extraction using 50% ethanol produced the highest yield of flavonoids. In turn, aqueous–methanol extracts were a better source of chlorogenic acid. No significant differences in terms of TPC and TFC were found between extracts prepared from hops and their pellets when water was used for extraction. Hydroalcoholic extracts, regardless of the type of alcohol and hop product, did not differ significantly in terms of antioxidant activities.

GORJANOVIĆ et al. (2013) determined the activity of individual compounds, prevalent hop phenolics, and bitter acids to obtain an insight into differences between results of antioxidant assays applied. By far superior H_2O_2 scavenging activity of humulone was followed by catechin, quercetin, xanthohumol, lupulone, and rutin. In contrast, DPPH scavenging activity of phenolics (quercetin > catechin > rutin > xanthohumol) was found substantially higher than activity of bitter acids. According to ferric reducing antioxidant power and scavenging of ABTS, higher antioxidant activity was ascribed to phenolics, while almost neglecting humulone. On the other hand, LIU et al. (2007) found that hop α-and β-acids and CO_2 hop extracts possess significant hydroxyl radical scavenging and antioxidant activities, furthermore, hop oil exhibited the highest hydroxyl radical scavenging activity, but lower antioxidant activity.

ŠIBALIĆ et al. (2020) reported the highest content of flavonoids (25.7 mg catechin equivalent/g) in extracts of hop variety Saaz. HPLC analysis confirmed that extract of hop variety Saaz has a higher amount of catechin (182.00 mg/ g) in comparison to Magnum and Hallertauer Tradition. The most abundant phenolic compounds detected in hop extracts were epicatechin gallate (68.9–357.2 mg/100 g), catechin (77.5–182.0 mg/100 g), p-hydroxyphenilacetic acid (19.3–55.9 mg/100 g), rutin (10.6–51.4 mg/100 g), myricetin (9.9–39.9 mg/100 g), and epicatechin (9.9–17.0 mg/100 g). Quercetin, caffeic acid, sinapic acid, p-hydroxybenzoic acid, p-coumaric acid and kaempferol were detected in concentration from 1.2 to 7.7 mg/100 g, while vanillic acid in concentration from 0.11 to 0.28 mg/100 g. OLŠOVSKÁ et al. (2013) found significant amounts of di-, tri- and tetramer proanthocyanidins consisting of (epi)catechin and (epi)gallocatechin in the hop samples. The dependence of the proanthocyanidin composition on both the variety and the growing locality was observed.

Regarding the **correlation between hop alpha acid content and phenolic content or antioxidant activity there is no consistent conclusion** in the literature. ŠIBALIĆ et al. (2020)

reported that hops share a similar pathway for polyphenol and alpha acid synthesis, meaning that low alpha varieties often have higher polyphenol contents and vice versa, they observed the highest polyphenol content for Magnum (the high alpha acid variety) rather than Saaz (the low alpha acid variety) in their study. This was confirmed by JERKOVIC et al. (2005) who claimed that the lower the alpha-acid content is, the higher the total stilbene content is. GUILLAUME et al. (2001) found that polyphenols are the main contributors to the reducing power of hops while α-acids do not contribute significantly to the reducing power. Similar results were reported by GORJANOVIĆ et al. (2013) that DPPH scavenging activity of phenolics was found substantially higher than activity of bitter acids. On the other hand, LIU et al. (2007) reported that the higher the hop bitter acids content is, the stronger the antioxidant activity is in the hop variety which is the opposite of the previous observations.

GUILLAUME et al. (2001) reported that hops contribute greatly to the reducing power of wort, the antioxidant activity of hops depends on the variety, quality, and processing. As revealed by nonenal potential measurements, pellet samples can prevent linoleic acid oxidation in the kettle, source of the cardboard flavor in aged beer. Furthermore, the experiments presented in the study of WIETSTOCK et al. (2010) demonstrated that longer heating times, or slower heating rates, prior to wort boiling, result in significantly higher radical formation in unhopped wort, and hop acids can substantially reduce their levels. α- and β-acids, possess the strong ability to reduce radicals in wort during boiling, thereby leading to a lower formation of staling aldehydes in beer. The isomerization of α-acids to iso-α-acids results in a loss of the hop acids' antioxidative power. Hop polyphenols appear to have a negligible role for the oxidative stability of wort as determined by using electron spin resonance spectroscopy, however they showed the highest activity to quench DPPH radicals. OLADOKUN et al. (2016) determined the phenolic acid and total polyphenol contents of beers, including dry-hopped ones, brewed in different geographical locations. The phenolic compound concentration was found to range from 3 to 12 mg/L and TPC was between 74 and 256 mg/L gallic acid equivalent, with the highest values identified in dry-hopped beers. They have found no linear relationship between total phenolic compound concentration and TPC.

3.8.7 Effect of technological steps during brewing on antioxidants

Polyphenols play very important roles in beer, from both nutritional and technological points of view (PANDEY et al. 2013). Technologically, they are involved in the formation of polyphenol-protein complexes that influence the colloidal stability of beer and the filterability, furthermore they are involved in the formation of color, taste, foam stability and through their antioxidant

properties, they are able to prevent the formation of sensorially undesirable compounds (DVORAKOVA et al. 2008; KARABÍN et al. 2013). Nowadays in general researchers are striving to avoid the haze formed by polyphenols and simultaneously to preserve other positive technological properties of these compounds (ARON et al. 2010).

There are studies investigating antioxidants from different aspects related to brewing. ZHAO (2015) investigated the effects of processing stages on the profile of phenolic compounds from barley to the final product. They found that the amount of phenolic compounds generally increased significantly during malting and mashing but decreased markedly during the subsequent fermentation and storage. Schwarz et al. (2012) studied how the mashing-in temperature influences the release of polyphenols. It was found that 40–45 °C is ideal for phenolic acid release from malt, while at temperatures above 65 °C no enzyme activity related to release of phenolic acids was detected. KROTTENTHALER et al. (2009) has reported that during mashing polyphenols undergo oxidation and polymerization processes, which result in the decrease of valuable antioxidants and consequently in a reduction of the taste stability. FUMI et al. (2011) studied polyphenols in all-malt worts and in maize adjunct worts, and their fate during the main brewing steps. They observed higher phenolic content in all-malt worts than in worts with maize adjunct, furthermore they reported that the overall brewing process reduces by 50% the initial content of total phenols.

PASCOE et al. (2003) studied the effect of critical stages of the brewing process on antioxidant activity and polyphenol content. They reported that the low molecular mass fraction (<5 kDa) was responsible for ~80% of the antioxidant activity levels of malt and beer samples. Decreases in antioxidant activity were observed after milling and beer filtration, but the decrease occurring after milling was not accompanied by a decrease in levels of phenolics. Increases were observed in levels of antioxidant activity after mashing, boiling, fermentation, chill-lagering, and pasteurization. Additional increase was observed after wort separation that was supported by increases in levels of most monitored phenolics. Total monitored polyphenols were reported to account for a large amount of the variation in antioxidant activity levels (measured by ABTS and FRAP assays), but are unlikely to be the only contributors to antioxidant activity levels.

In a recent study ŠIBALIĆ et al. (2020) analyzed phenolic compounds during the brewing of two types of pale lager beers produced at industrial scale according to German Beer Purity Law using the same brewing procedure, but different hop varieties. Their aim was to analyze simple phenolic compounds, total polyphenols and total flavonoids content, antioxidant activity in raw materials (malt and hops) and in samples taken out from several production stages (after wort production, boiling, fermentation, filtering, and pasteurization). Eighteen different phenolic

compounds which belong to the groups of hydroxybenzoic and hydroxycinnamic acid derivates, flavan-3-ols, flavonols and procyanidin dimers were analyzed. Among all tested phenolic compounds, 9 compounds were identified in malt, 13 in hops, while 10 in samples taken from the brewing process. They concluded that the antioxidant power of beer greatly depends on the variety of hops added during boiling. They have found that boiling and pasteurization are the crucial phases for the degradation of polyphenols and flavonoids.

4. MATERIALS AND METHODS

The materials and methods chapter can be divided into three parts based on the methods. The first part discusses materials and methods related to the measurement of folate content, antioxidant activity, and total phenolic composition. The second part contains materials and methods that are related only to antioxidant activity and total phenolic composition. In the third part, the materials and methods are related to the determination of targeted phenolic compounds, total flavonoid content, total phenolic content and DPPH radical scavenging activity.

In case of total phenolic composition all the phenolic compounds of a sample based on the HPLC chromatograms were tentatively identified based on their retention times and spectral characteristics. Targeted phenolic compounds are determined by HPLC using standards while total polyphenol content refers to the spectrophotometric method TPC.

4.1 Materials and methods used for the determination of folate content, antioxidant activity and total phenolic composition.

The materials and methods used for sample preparation discussed in this chapter are the same for folate, antioxidant activity and total phenolic composition measurements.

4.1.1 Malts

To investigate the folate content, antioxidant activity and total phenolic composition of different malts, three types of barley malts (Pilsner, Caramel, Coloring), wheat, rye, oat, einkorn, emmer, and spelt malts were analyzed. The barley malts, wheat, rye and oat malts were purchased from Weyermann malting company (Bamberg, Germany), while the einkorn, emmer and spelt malts were produced on a Schmidt-Seeger micromalting plant (Bühler AG) at the university.

4.1.2 Malting

For the malting experiments two malting barley varieties have been selected, the spring variety was Etincel, and the winter variety was Casanova. Both varieties have been malted on micro- and industrial scale. The barleys for micro- and industrial scale malting are of the same variety, but have been harvested in successive years. The micro scale and industrial scale maltings were carried out from the barleys cropped in 2017 and in 2018, respectively.

The micro scale malting was carried out on a Schmidt-Seeger micromalting plant (Bühler AG). The industrial scale malting was carried out in an industrial malting factory. The same malting technology was applied on both micro- and industrial scale. Both the technology and the time of sampling is shown on Figure 12. The abbreviation of sample names are as follows: B - barley, AS - after steeping, G 1-4 - germination days 1-4, M – malt.

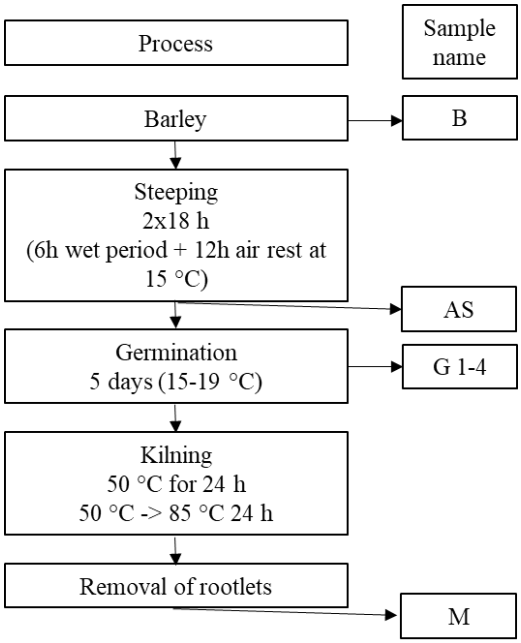


Figure 12: Micro- and industrial scale malting technology and time of sampling

4.1.3 Roasting experiment of malts

The roasting was carried out in a hot air fryer. On the false bottom 100g malt was placed and air was circulated through the malt bed for different time periods at different temperatures: for 20 minutes at 100 °C, 125 °C, 150 °C, 175 °C and at 200 °C for 5, 10, 15, 20 minutes.

4.1.4 Extended protease rest

To study the effect of protease enzymes during mashing on the investigated components and parameters, an extended protease rest was applied. The mashing in temperature was 52 °C and the water: malt ratio 4:1. The mash was kept for 40 minutes at 52 °C in a 1-CUBE mashing bath (1-CUBE s.r.o., Czech Republic) and samples were taken after every 10 minutes. The samples were immediately cooled down under 10 °C to stop all the enzymatic processes, centrifuged at 10 000 rpm and the supernatant was put in the freezer until analysis.

4.1.5 Brewing technology of pale wort and beer

To study the evolution of the selected parameters during the brewing process, worts were produced with different equipment on different scales: laboratory- and pilot scale. On laboratory scale a wort was produced with infusion technology in a 1-CUBE mashing bath (1-CUBE s.r.o., Czech Republic). On laboratory scale the process ended after cooling the hopped wort because fermentation similar to industrial or pilot conditions is not feasible. On pilot scale the same infusion technology was applied as on laboratory scale but was carried out on a 50l capacity pilot brewery with steam heating. The brewery is consisting of a mash tun, lauter tun, boiling kettle, whirlpool, plate heat exchanger and unitanks for fermentation. On pilot scale the process ended at the end of lagering. For the brewing experiments of the pale wort and beer 100% Pilsner malt was used.

4.1.5.1 Laboratory scale brewing of pale wort

The brewing technology, the grain bill, and the time of sampling under laboratory conditions is shown on Figure 13. The abbreviation of sample names are as follows: M 52 °C – end of 52 °C rest during mashing, M 62 °C – end of 62 °C rest during mashing, M 72 °C – end of 72 °C rest during mashing, FW – First wort, HW – Hopped wort. At the beginning of the α-amylase rest 100ml distilled water (DW) was added to the mash to substitute the sparging step as this cannot be carried out properly on laboratory scale. The temperature between the enzymatic rests was raised by 1 °C/min. The filtration was carried out using Whatman MN-615 filter paper (GE Healthcare). The hop boiling was carried out in Erlenmeyer flask, in the 5th min 0.72 g Hallertau Tradition hops (10% a-acid) were added to get a bitterness value of 20 International Bitterness Units (IBU). Samples for analysis were taken at the end of all enzymatic rests during mashing, after filtration and after cooling. The samples were immediately cooled down under 10 °C to stop all the enzymatic processes, centrifuged at 10 000 rpm and the supernatant was put in the freezer until analysis.

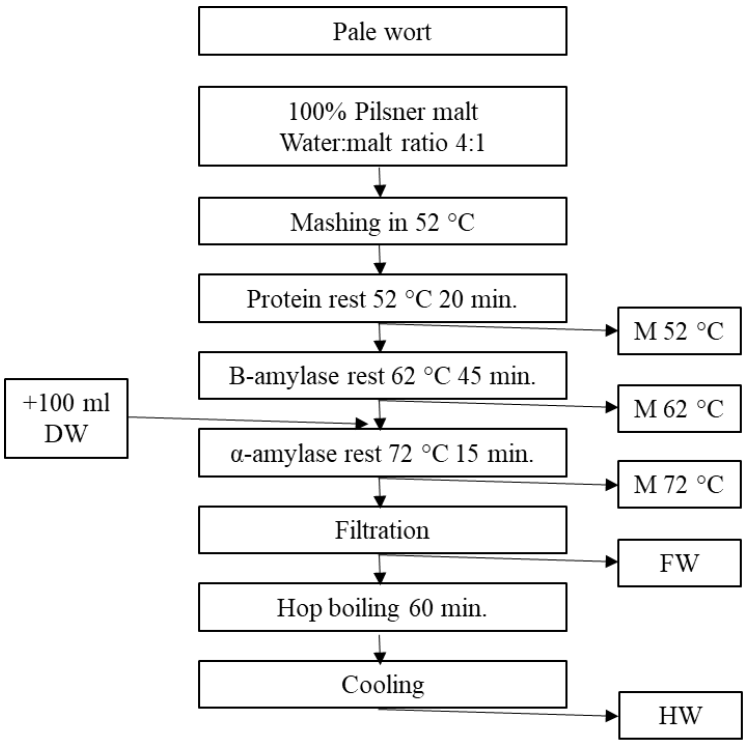


Figure 13: Brewing technology and time of sampling of laboratory scale pale wort

4.1.5.2 Pilot scale brewing of pale beer

The pilot scale brewing was carried out on a 50-liter capacity pilot brewery with steam heating. The brewing technology, the grain bill and the time of sampling on pilot scale is shown on Figure 14. The abbreviation of sample names are as follows: M 52 °C – end of 52 °C rest during mashing, M 62 °C – end of 62 °C rest during mashing, M 72 °C – end of 72 °C rest during mashing, FW – First wort, S1 – First sparging, S2 – Second sparging, SW – Sweet wort, HW – Hopped wort, MF 1-5 – Main fermentation days 1-5, L 1-3 – Lagering weeks 1-3. The temperature between the enzymatic rests was raised by 1 °C/min. The hop boiling of the sweet wort lasted for 60 minutes, in the 5th min 30 g Hallertau Tradition hops were added to get a bitterness value of 20 IBU. For the fermentation SafAle US-05 yeast (Fermentis) was used. Samples were taken at the end of all enzymatic rests during mashing, after first wort separation, from each sparging, from the sweet wort before hop boiling (first wort+ 2 spargings together), after cooling, every day of the main fermentation and every 7th day of the lagering. The samples were taken and stored the same way as in case of laboratory scale brewing.

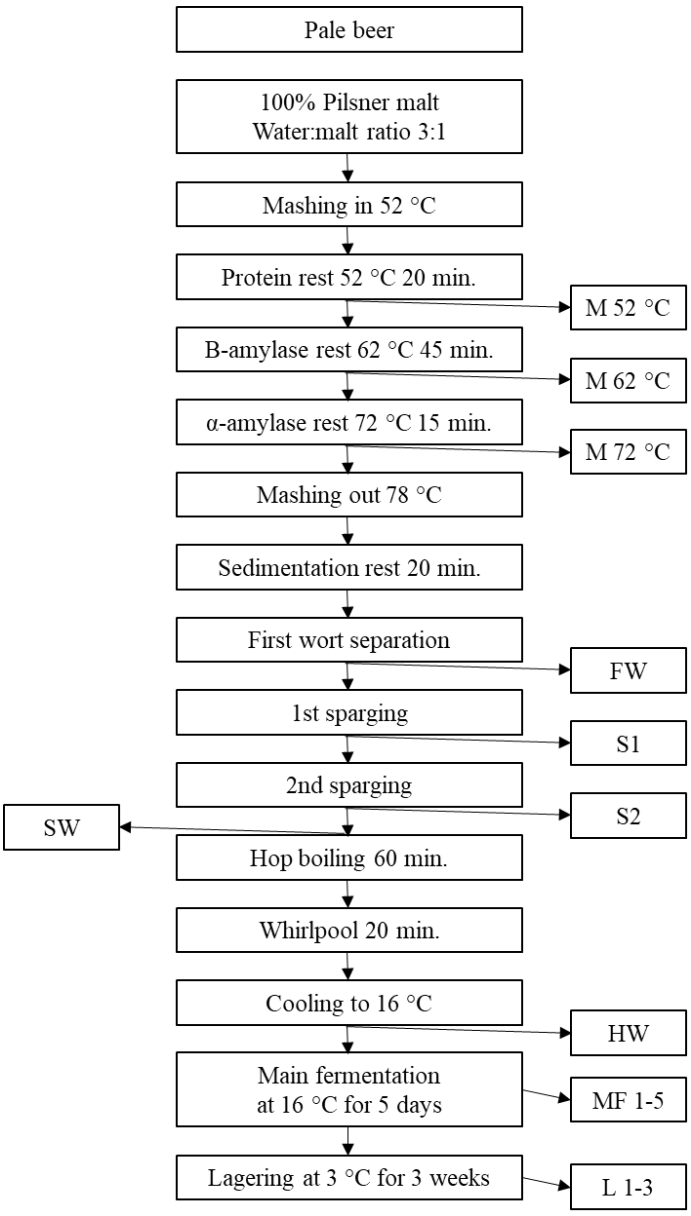


Figure 14: Brewing technology and time of sampling of pilot scale pale beer

Beers were produced with the same technology and on the same scale as in case of the pale beers, only the malt composition changed. For the brewing experiments to investigate the evolution of antioxidant activity and total phenolic composition the grain bill included 70% Pilsner malt, 10% Munich malt, 10% Cara Munich malt, 7% Cara Bohemian malt and 3% Chocolate. The Chocolate malt was added when the mash reached 72 °C to achieve a less intense roasted flavor, highlighting dark chocolate notes.

4.1.6 Brewing technology of dark wort and beer

4.1.6.1 Laboratory scale brewing of dark wort

The brewing technology, the grain bill, and the time of sampling under laboratory conditions are shown on Figure 15. The abbreviation of sample names are as follows: M 52 °C – end of 52 °C rest during mashing, M 62 °C – end of 62 °C rest during mashing, M 72 °C – end of 72 °C rest during mashing, FW – First wort, HW – Hopped wort. At the beginning of the α-amylase rest 100ml distilled water (DW) was added to the mash to substitute the sparging step as this cannot be carried out properly on laboratory scale. The temperature between the enzymatic rests was raised by 1 °C/min. The filtration was carried out using Whatman MN-615 filter paper (GE Healthcare). The hop boiling was carried out in Erlenmeyer flask, in the 5th min 0.58 g Fuggles hops (5.2% α-acid) were added to get a bitterness value of 20 IBU. Samples for analysis were taken at the end of all enzymatic rests during mashing, after filtration and after hop boiling. The samples were immediately cooled down under 10 °C to stop all the enzymatic processes, centrifuged at 10 000 rpm and put in the freezer until analysis.

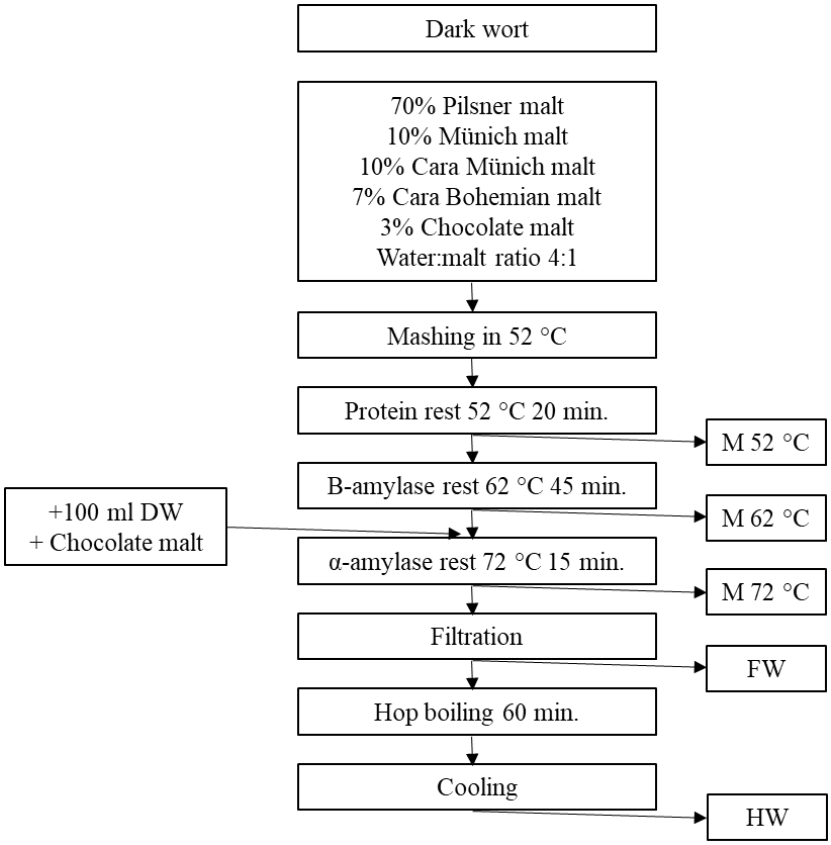


Figure 15: Brewing technology and time of sampling of laboratory scale dark wort

4.1.6.2 Pilot scale brewing of dark beer

Pilot scale brewing was carried out on a 50-liter capacity pilot brewery with steam heating. The brewing technology and the time of sampling on pilot scale is shown on Figure 16. The abbreviation of sample names are as follows: M 52 °C – end of 52 °C rest during mashing, M 62 °C – end of 62 °C rest during mashing, M 72 °C – end of 72 °C rest during mashing, FW – First wort, S1 – First sparging, S2 – Second sparging, SW – Sweet wort, HW – Hopped wort, MF 1-5 – Main fermentation days 1-5, L 1-3 – Lagering weeks 1-3. The temperature between the enzymatic rests was raised by 1 °C/min. The hop boiling of the sweet wort lasted for 60 minutes, in the 5th min 60 g Fuggles hops were added to get a bitterness value of 20 IBU. For the fermentation SafAle US-05 yeast (Fermentis) was used. Samples were taken at the end of all enzymatic rests during mashing, after first wort separation, from each sparging, from the sweet wort before hop boiling (first wort+ 2 spargings together), after cooling, every day of the main fermentation and every 7^{th} day of the lagering. The samples were taken and stored the same way as in case of laboratory scale brewing.

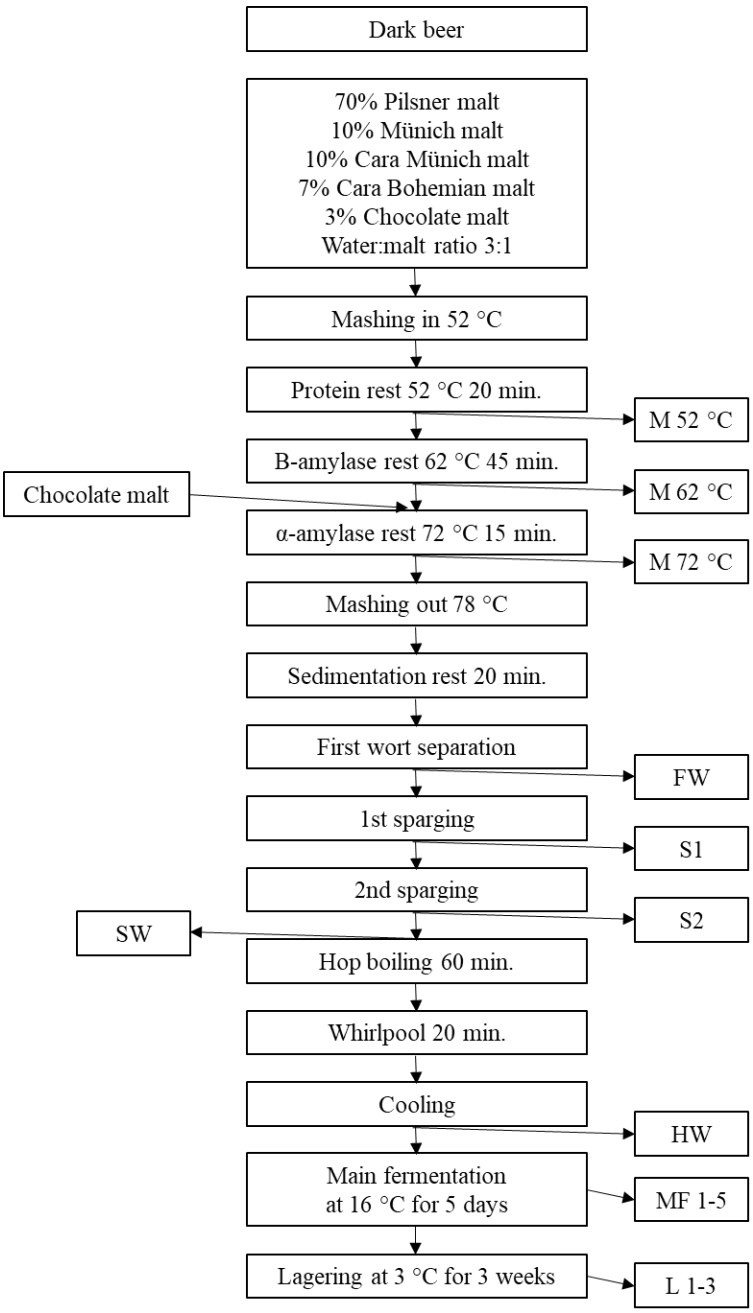


Figure 16: Brewing technology and time of sampling of pilot scale dark beer

Materials and methods used for the determination of antioxidant activity and total phenolic composition

In this section materials and methods are discussed which were used only for the determination of antioxidant activity and total phenolic composition.

4.7 Hops

For the determination of antioxidant activity and phenolic composition 16 hops were included. The names and α-acid contents of the samples are shown in Table 2, all the hops are from USA Hops (Yakima, USA).

Table 2: Name and α-acid content of hop samples

Name	α-acid content [%]	Name	α-acid content [%]
Denali	15.4	**Tahoma**	6.9
Chinook	11.6	**Crystal**	4.9
Sorachi Ace	12.9	**Azacca**	14.4
Pekko	16.8	**Mt. Hood**	4.0-6.5
CTZ	15.7	**Comet**	6.4
Centennial	8.9	**Nugget**	15.5
Belma	10.7	**Columbus**	17.4
Cashmere	7.1	**Willamette**	4.8

4.8 Materials and methods used to determine targeted phenolic compounds, total flavonoid content, total phenolic content, and DPPH radical scavenging activity

4.8.1 Congress mashing with adjuncts and special malts

To investigate the effect of adjuncts and special malts on targeted phenolic compounds and total flavonoid content, total phenolic content and DPPH radical scavenging activity, congress worts were prepared according to EBC Analytica method 4.5.1. Adjuncts were applied to a level of 50% w/w added to the Pilsner malt, while caramel and roasted malts were applied in smaller amounts, 10% w/w and 5% w/w respectively, as used in usual brewing practice. Furthermore, congress worts were also prepared from 100% barley grist with the addition of special malts to determine whether there was any effect of enzymatic activity of Pilsner malt on the phenolic

composition and antioxidant activity during mashing. The names and compositions of the samples are shown in Table 3.

Table 3: Grain composition and extract content of the congress worts

Sample name	Grain bill	Extract content [%m/m]
Pils	100% Pilsner malt	8.34
Pils+barley	50% Pilsner malt + 50% Barley grist	7.85
Pils+rice	50% Pilsner malt + 50% Rice flour	5.68
Pils+corn	50% Pilsner malt + 50% Corn flour	7.46
Pils+wheat	50% Pilsner malt + 50% Wheat grist	8.06
Pils+caramel	90% Pilsner malt + 10% Caramel malt	8.08
Pils+roasted	95% Pilsner malt + 5% Roasted malt	8.34
Barley	100% Barley grist	1.69
Barley+caramel	90% Barley grist + 10% Caramel malt	2.14
Barley+roasted	95% Barley grist + 5% Roasted malt	1.76
Caramel	100% Caramel malt	4.19
Roasted	100% Roasted malt	7.76

4.8.2 Comparison of infusion and decoction mashing

The mashing experiments were carried out in a full automated pilot brewery with 100l capacity at the University of Chemistry and Technology (Prague). Worts were produced with three different mashing methods, two decoction and one infusion technology. The grain bill was the same for all mashings: 77% Pilsner malt, 12% Cara Red, 8% Melanoidin, 3% Cara Münich III. Mashing in was carried out with 70 l water, the water: malt ratio was 4:1. In case of Decoction 1 one third of the mash was separated for boiling while in case of Decoction 2 mashing it was two third. Wort sampling was performed after lautering from the first wort, after sparging from the second wort and from the sweet wort before hop boiling (first+second wort together). The mashing diagrams are shown on Figure 17.

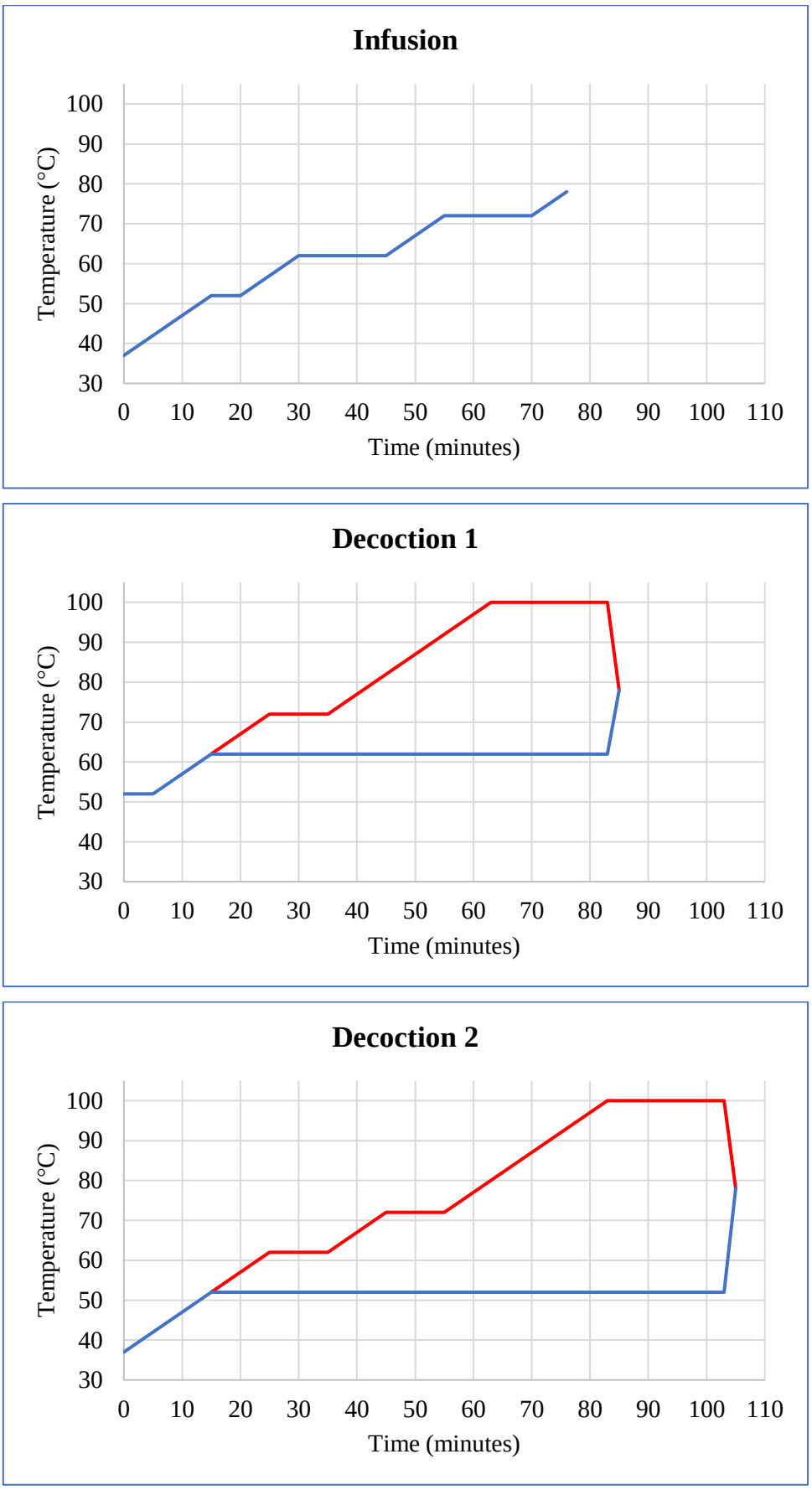


Figure 17: Mashing technology of worts

4.8.3 Comparison of high gravity brewing and conventional brewing

To compare the effect of high gravity brewing and conventional brewing on targeted phenolic compounds, total flavonoid content, total phenolic content, and DPPH radical scavenging activity, two beers were brewed with exactly the same technology and recipe but with different original extract content. One had 8.84%m/m original extract (Beer 9) and the other 13.98%m/m original extract (Beer 14). Before bottling Beer 14 was diluted with oxygen-free, carbonated water to have an original extract content identical to Beer 9.

4.9 Analytical methods

4.9.1 Determination of folate content

During my research, the microbiological method was used because it is suitable for the determination of the total folate content, does not require sophisticated instrumentation, is relatively economical and has high sensitivity (PFEIFFER et al. 2009).
For the determination of total folate content, a microbiological microtiter plate from R-Biopharm AG (Darmstadt, Germany) was used (AOAC-RI – 100903). The procedure was the following: exactly 1 g grinded or 1 ml liquid sample and 20 mg pig pancreatin were weighed into a 50 ml centrifuge vial, then 40 ml phosphate buffer (0.05 mol ascorbate, pH 7.2) was added and shaken. It was incubated for 2 hours at 37 °C in dark and shaken 5 times during the incubation. Thereafter, the extract was heated for 30 minutes at 95 °C in a water bath, chilled down quickly to below 30 °C, and centrifuged at 8000 x g for 5 minutes. Finally, 150 µl folic acid assay medium and 150 µl standard or diluted sample were pipetted into the wells covered by *Lactobacillus rhamnosus*. It was incubated at 37 °C in the dark for 48 hours, and optical density was measured at λ=600 nm. The measurement was carried out in three parallels. All the results are given in µg/100g dry matter (d.m.).

4.9.2 Determination of antioxidant activity

The antioxidant activity was determined by five commonly applied assays as there is no standard method which can objectively characterize this parameter. The following assays were applied because these are widely used to determine this parameter so there is a possibility to compare our results with others'. Furthermore, these assays are easily reproducible, however are not selective

to certain components, these methods are determining the reducing ability of the sample. (HUANG et al. 2005) All the results were expressed as mg/100g or mg/100cm^3 ascorbic acid equivalent (AAE), in case of grains related to dry matter (d.m.). All the measurements were carried out in three parallels.

ABTS radical scavenging activity

The assay was performed as described by RE et al. (1999). 10 µl degassed sample was pipetted into 96 well plates. 20 µl solution was added, which contained 9% NaCl, 1% glucose, 50 mg/mL myoglobin dissolved in pH 7.4 potassium-phosphate buffer. Then 150 µl 1 mg/mL 2,20- Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) solution and 25 µl 3% H_2O_2 dissolved in 0.1 M pH 5 citric buffer were added. It was shaken for 15 min at 37 °C then absorbance was measured at λ = 405 nm.

Ferric reducing antioxidant power

The assay was performed according to BENZIE et al. (1996). Samples were added to FRAP reagent that contained 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) dissolved in 40 mM HCl, 300 mM pH 3.6 acetate buffer and 20 mM $FeCl_3$*$6H_2O$. After 5 min of incubation time, absorbance was measured at λ = 593 nm.

Total polyphenol content

The assay was performed based on the description of SINGLETON et al. (1965), which is based on the reduction power of antioxidants rather than on the selective reaction of polyphenols, thus it was evaluated together with the other antioxidant activity assays (MARTINEZ-PERINAN et al. 2011). First 1250 µl ten-fold diluted Folin-Cioalteau reagent and 240 µl methanol: water (4:1) solvent were pipetted in the test-tubes. Then 10 µl degassed sample was added. After homogenization and 1 min reaction time 1 cm^3 0.7 M Na_2CO_3 was added, vortexed and before measurement the mixture was allowed to stand for 5 min at 50 °C. The absorbance was measured at λ = 765 nm.

Cupric reducing antioxidant capacity

The assay was performed according to APAK et al. (2004). 100 µl sample was added to 1 cm^3 10^{-2}M $CuCl_2$, 1 cm^3 7.5*10^{-3}M neocuproine solution (dissolved in 96% ethanol), 1 cm^3 pH 7.4 1 M NH_4Ac buffer solution and 0.9 cm^3 distilled water. It was incubated in dark for 30 min and the absorbance was measured at λ = 450 nm.

DPPH radical scavenging activity

The assay was performed as described by BRAND-WILLIAMS et al. (1995). $6*10^{-5}$M 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution was prepared with methanol. 100 µl sample was added to 3.9 cm^3 DPPH solution and was incubated in dark for 20 min then the absorbance was measured at $\lambda = 517$ nm.

Total flavonoid content

The total flavonoid content (TFC) was determined based on the description of GRANATO et al. (2011) using a colorimetric method employing aluminium chloride. 1 ml distilled water and 60 µl 0.5M $NaNO_2$ were added to 125 µl sample. After 5 minutes reaction time 60 µl $AlCl_3$ was added which was followed by 5 minutes reaction time again. Finally, 400 µl 1M NaOH was added, vortexed and absorbance was determined at λ=510 nm. The flavonoid content was determined using a standard curve of catechin and the results were expressed as milligrams of catechin equivalents per liter of sample (mg CE/l). Data presented are the average of three measurements.

4.9.3 Determination of total phenolic composition by HPLC-DAD

To extract phenols, the solid samples (grains, malts, hops) were ground and homogenized by a laboratory homogenizer. The ground samples, 4 g of each, were transferred to an Erlenmeyer flask with the addition of 30 mL of the mixture of 75% methanol + 23% distilled water + 2% acetic acid. Thereafter the mixture with the sample was subjected to ultra-sonication in an ultrasonic water bath for 4 minutes. Then the sample was shaken by a mechanical shaker for 15 minutes. The mixture was kept overnight at 4 °C, then filtered through a filter paper. It was further cleaned-up by passing through a 0.22 mm PTFE syringe filter before injection on to the HPLC column for the analysis of phenolics. Liquid samples (wort, beer) were diluted and filtered through a 0.22 mm PTFE syringe filter into HPLC vials. Nucleosil C18, 100-3, Protect-1, 250 * 4.6 column (Macherey-Nagel, Duren, Germany) was used to separate phenolic compounds using a gradient elution of 1% formic acid in water (A) and acetonitrile (B), with a flow rate of 0.6 mL/min. Gradient elution started with 2% B; it changed in 10 min to 13% B, then in 5 min to 25% B, and then in 15 min to 40% B, and finally, in 7 min it turned to 2% B. The phenolic compounds were tentatively identified based on their retention times and spectral characteristics found in the literature and based on own experiences (ARAPITSAS 2008, LONG-ZE LIN 2008, LEITAO 2012, CARVALHO 2015, QUIFER-RADA 2015, STEFANELLO 2018, QUAN 2018,

ANDREASEN 2000). The different compounds were quantificated as rutin equivalent (RE), after taking the absorbance values at the maximum wavelength of each compound.

4.9.4 Determination of targeted phenolic compounds by HPLC-DAD

Solid-phase extraction

All wort samples were purified by solid phase extraction (SPE) prior to HPLC analysis. For purification, StrataTM-X 33 µm Polymeric Reversed Phase 200mg/6 ml (Phenomenex) cartridges were used. The cartridges were conditioned with 5 ml of methanol followed by 10 ml of distilled water. An aliquot of the wort sample (50 ml) was passed through the cartridges. Subsequently, phenolic compounds were eluted with 5 ml of acidic methanol (0.1 v/v% orthophosphoric acid). The organic eluate was evaporated to dryness under vacuum at 35°C. The residue obtained was dissolved in 1 ml of acidic methanol (0.1 v/v% orthophosphoric acid) and filtered through a polytetrafluoroethylene (PTFE) syringe filter, 0.45 µm, before transferring to the HPLC vial.

Analysis of phenolic compounds

The concentration of phenolic monomers was determined using an Agilent 1100 HPLC system (Agilent Technologies, USA) equipped with quaternary pump and diode array detector. Twenty µL of sample were injected onto a Kinetex 5u Phenyl-Hexyl 100A HPLC column (5 µm; 150 x 4.6 mm) at 30 °C with a mobile phase flow rate of 0.8 ml/min. The mobile phase A was degassed gradient grade acetonitrile acidified with 85 % orthophosphoric acid (100:0.03 v/v) and the mobile phase B was ultrapure water acidified with 85 % orthophosphoric acid (100:0.03 v/v). The gradient elution was set as follows: 0-20 min, 100-90 % B; 20-40 min, 90-70 % B; 40-45 min, 70-0 % B and 45-50, 0-100 % B. Detection of phenolic monomers was carried out at three wavelengths near the absorbance maximum of the compounds analyzed (253, 280 and 320 nm). Quantification was carried out by external calibration using calibration curves of standard solutions. Calibration curves were constructed for the following phenolic compounds: 4-hydroxybenzoic acid, trans-ferulic acid, (+)-catechin, (-)-epicatechin, procyanidin B3 (Sigma-Aldrich) and vanillic acid (Honeywell Fluka™).

4.9.5 Extract content

The determination of extract content was carried out using an Anton Paar Beer Analyzer System.

4.9.6 Moisture content

The moisture content of the barley and malt samples was determined by an AND MX-50 Moisture Analyzer.

4.10 Statistical analysis

The Pearson's correlation matrix for determining the correlation between the properties of hops and One-Way Anova to test differences between the properties of high gravity brewed and traditional beer were carried out in IBM SPSS Statistics 25 software.

5. RESULTS AND THEIR DISCUSSION

5.1 Evolution of folate content from grains to malt

As a preliminary experiment, the folate content of special barley malts and malts made from different cereals was examined to see what folate content they have compared to Pilsner malt, the most commonly used malt for brewing. In addition to Pilsner malt special barley malts (caramel, coloring), wheat malt, rye malt, oat malt and the less commonly used einkorn, emmer and spelt malts were studied.

5.1.1 Folate content of malts made from different cereals

As it can be seen on Figure 18 there are magnitude differences between the different types of barley malts. Pilsner type barley malt, which is an enzyme rich pale malt, kilned around at 80-85 °C, has the highest folate content (44.7 µg/100g d.m.). Caramel malt which is usually used to give amber-brownish color and caramel aroma to beer has a folate content of 5.5 µg/100g d.m. that is due to the high temperature (140-180 °C) at this type of malt is roasted to caramelize the sugars in the endosperm. Coloring malt does not contain any folate as it is roasted above 200 °C, at which temperature folate is completely degraded.

Wheat, rye and oat malts have similar folate contents. In case of wheat and rye these results are due to the lack of husk as the outer layers and the husk are richer sources of folate than the endosperm of the kernels (EDELMANN et al. 2013). In case of oats, the kernel has a large husk but despite this, based on my results, this does not result in a higher folic acid content. Perhaps the higher proportion of husk in terms of folate content is just as disadvantageous as the lack of husk, barley husk is smaller than oats, higher than wheat and rye, which may be related to folate content. This result agrees with the observation of EDELMANN et al. (2012) who reported lower folate concentration in oats than in barley.

Einkorn, emmer and spelt are rarely used ingredients in brewing, rather they can be considered as alternative raw materials. It is shown that einkorn malt and emmer malt have lower folate content while spelt malt is similar to wheat, rye and oat malts. However, einkorn was malted in its hulled form while emmer and spelt in their hull-less forms.

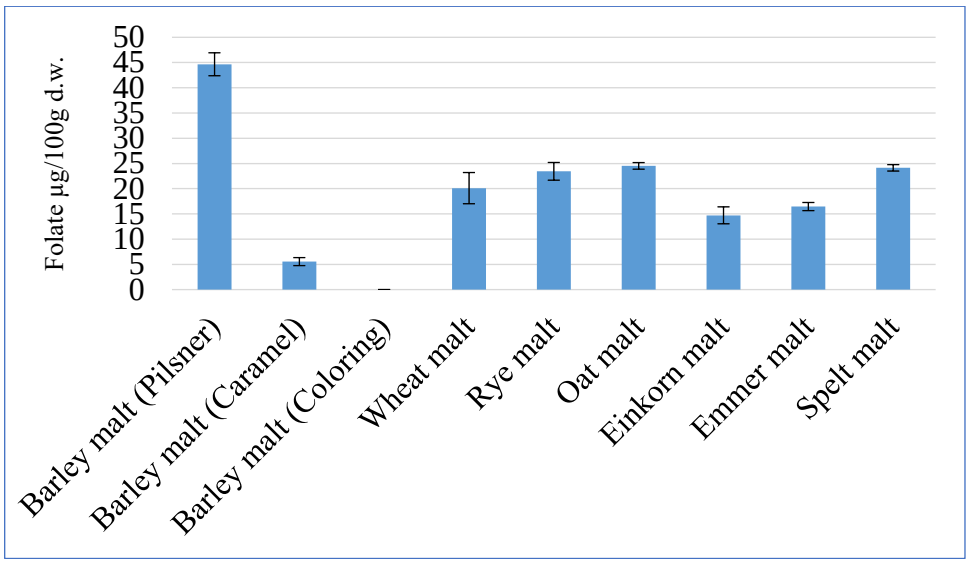


Figure 18 Folate content of malts (mean, sd, n=3)

5.1.2 Evolution of folate content during malting on micro and industrial scale

To obtain information on the evolution of folate content during malting, malts were prepared on micro and industrial scale with the same malting parameters. The same spring and winter barley varieties were used for malting, but they were cropped in successive years. Before studying the processes that take place during malting the folate content of the barleys were analyzed.

The evolution of folate content during the malting of the spring and winter barleys cropped in 2017 and 2018 is shown on Figure 19. It can be observed in these Figures that there are considerable differences between barleys of the same variety but different harvest years. Differences of the folate content of the same barley varieties cropped in successive years were also observed by EDELMANN et al. (2013) who reported that environmental factors might also cause variations in folate content even regarding samples of the same cultivars.

Regarding the malts produced from the barleys, in case of both micro- and industrial scale production the malts of winter cultivars have higher folate content than malts of the spring ones. The micro-scale malt of spring barley has a folate content of 78.4 while the winter barley 113.3 µg/100g d.m.. Concerning industrial malting the results are 94.3 for spring cultivar and 109.8 µg/100g d.m. for the winter cultivar. The higher folate content of the winter cultivars could be explained by the thicker husk of winter barleys, as folate accumulates mainly in the outer layers of the kernel and in the germ, thus the thicker husk can function as a protective layer (GIORDANO et al. 2016).

During the malting process with respect to both micro- and industrial scale malting the folate content increased. The folate content during micro-scale malting of the spring barley increased by almost 8-times while that of the winter barley by 6.5-times. Regarding industrial malting 4-times increase was observed in case of spring barley and 7-times in case of the winter one. It is due to the folate synthetization of the germ during germination, as it is essential for growth and cell differentiation (JÄGERSTAD et al. 2005). Furthermore, it maybe due to the enzymatic processes which can release folate from its bound form. In the only scientific publication available about the changes of folate content during malting, WALKER (2003) observed a 4-times increase which equals to the lowest increase observed by me in case of the industrial malting of the spring barley cultivar.

There is a very similar trend how folate content changes during the micro-scale malting of both barleys. After a continuous increase it reaches a peak on the 3rd day of germination (spring barley: 112.5, winter barley: 138.1 µg/100g d.m.) and shows a 15-20% decrease during kilning. This trend is similar to that published by WALKER (2003).

The loss of folate in the final malt (after kilning) compared to the green malt (the malt at the end of germination, before kilning) in case of micro scale malting can be explained by two reasons. One is the degradation of folate due to heat treatment. According to WALKER (2003) folate synthesized during germination by the embryo is not stabilized by binding to proteins and may therefore be more vulnerable to heat. The other is the removal of the rootlets after kilning. In case of malts produced for brewing the removal of the rootlets after kilning is an essential step as rootlets have a relatively high oil content which is undesirable in brewing. But rootlets are a rich source of folate thus it could cause the decrease of the vitamin content in case of the final malt compared to the green malt.

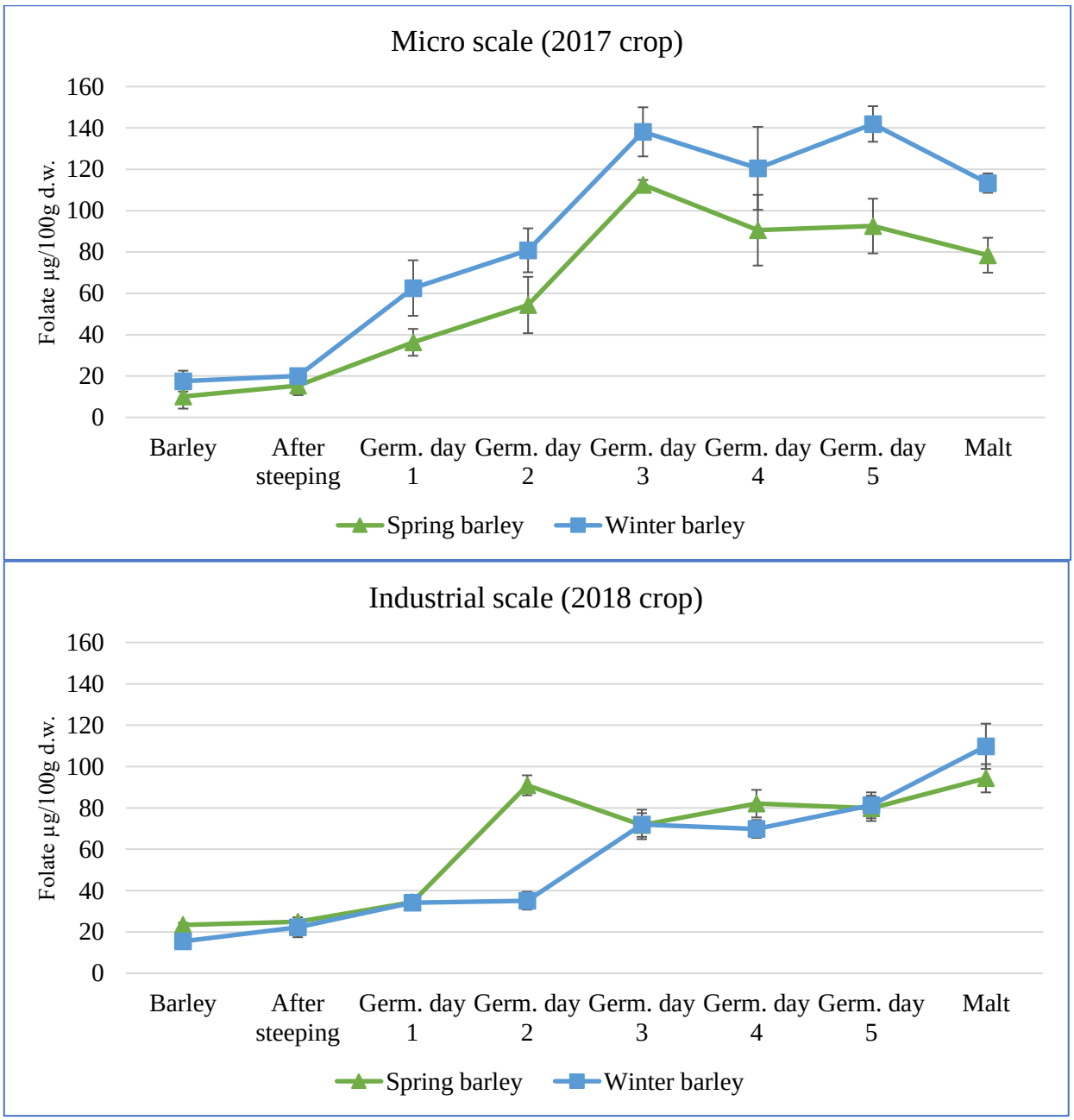


Figure 19: Evolution of folate content during micro and industrial scale malting (mean, sd, n=3)

Change in folate content of samples during germination shows similar trends in micro and industrial scale malting. The spring cultivar has a peak on the 2nd day of germination and the folate content of the final malt is only slightly different from this result. Regarding the winter barley, there was a greater increase between the 2nd and the 3rd day of germination which was followed by stagnation of folate content until kilning then another increase was observed during the kilning process. The increase of folate content by 18-35% during the kilning of industrial malts was unexpected, since during micro-scale malting decrease was observed at this stage of the process. As the same temperatures and times were applied in both micro and industrial scale, the different trends during kilning cannot be explained by a scientifically substantiated fact. The

varying degrees and methods of removal of the rootlets in laboratory and in the industry could affect folate content but that probably will not cause that much of a difference.

Based on the evolution of folate content malting can be divided into two stages. There is a formation phase that is well observed at both micro and industrial scales and is caused by the fact that folate is produced by the embryo because it is required for growth and cell differentiation. It is an internal, gene-dictated process, it does not affect the tendency of production scale. It is affected by its extent, because the controllability of parameters is different in micro and industrial scales, and the production volume also affects how much heat or moisture each grain receives.

In the second stage, the change in the amount of folate is determined only by an external effect, namely heat treatment. Here only this external influence affects, not only the extent but also the trend.

5.1.3 Effect of roasting on folate content

In order to study the effect of the heat treatment in more depth, roasting experiments were carried out. Large number of studies have been performed on the effect of processing on folate content of different foods, and it was proven that the food matrix highly influences folate degradation (DE SOUZA et al. 1986, MELSE-BOONSTRA et al. 2002;).

For the roasting experiment Pilsner malt, produced on micro-scale, was used. In the first experiment the malt was treated at different temperatures (100 °C, 125 °C, 150 °C, 175 °C) for 20 minutes in each case (Figure 20). The results obtained show that roasting for 20 minutes at 100 °C does not cause a decrease in folate content. This may be due to the fact that, as mentioned above, the unstable folate (which is produced by the embryo during germination) degrades during kilning, and the folate in the final malt, which is structurally bound in the kernel, found in polyglutamate form bound to proteins, is more stable to heat (WALKER 2003). Thus, the Pilsner malt that was involved in the roasting experiment probably had a more thermostable folate content because it had already been kilned: the unstable folate had already been degraded, and the malt contained the more thermostable, structurally bound folate. With increasing temperature, the folate content decreases with linear tendency. The folate content does not change significantly after the treatment for 20 minutes at 100 °C, but due to the treatment at 125, 150, and 175 °C it decreases linearly. MIFTAKHUSSOLIKHAH et al. (2015) reported similar result in case of mung bean flour, when due to roasting at 160 °C for 15 minutes the folate content decreased to less than one third.

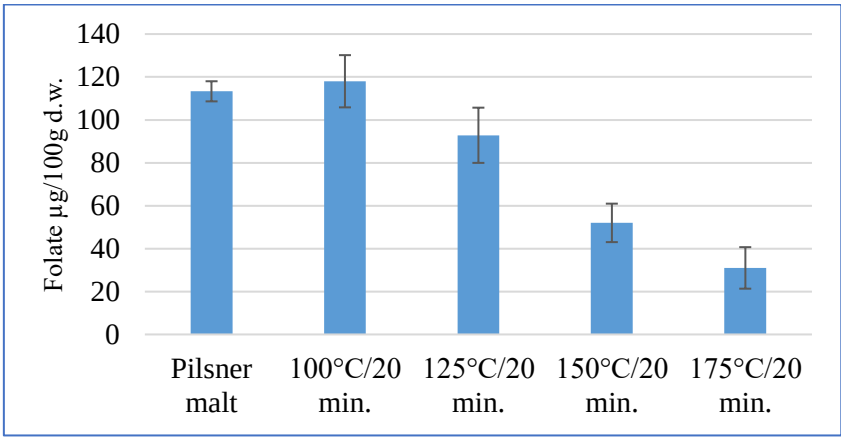


Figure 20: Effect of roasting on the folate content of Pilsner malt at different temperatures for 20 minutes (mean, sd, n=3)

Afterwards, the roasting was carried out at 200 °C for different periods of time (5, 10, 15, 20 minutes) (Figure 21). This temperature had a great effect on folate content, only 5 minutes at 200 °C decreases the folate content to about one third, and in 20 minutes folate is completely degraded. These results indicate that special malts that are treated above 100 °C for different time periods suffer from different degrees of folate loss. It can be stated that with increasing color of the malt the folate content decreases.

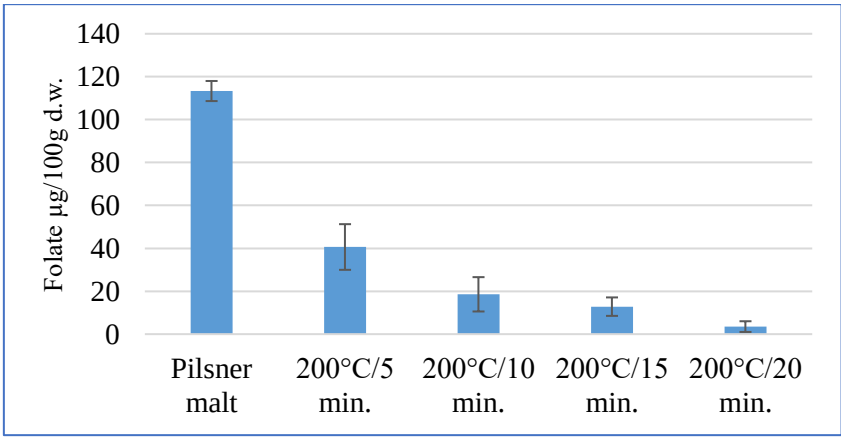


Figure 21: Effect of roasting on the folate content of Pilsner malt at 200°C for different time periods (mean, sd, n=3)

5.2 Effect of brewhouse processes on the evolution of folate content

After studying the evolution of folate content during malting, the effect of brewhouse processes was investigated. Among the brewhouse processes, mashing can be modeled in laboratory conditions. A preliminary experiment with extended protein rest was followed by the comparison of the change of folate content during laboratory and pilot scale mashing.

5.2.1 Evolution of folate content during extended protease rest

As folate occurs in grains mainly bound to proteins, in polyglutamate form (GIORDANO et al. 2016), an experiment was carried out by the extension of the protease rest to see if it has any effect on the liberation of folate. The mash was kept at 52 °C for 40 minutes and samples were taken after every 10 minutes. The results showed (Figure 22) no effect of the extended rest on folate content. It is maybe due to the fact that malts which are available nowadays are very well modified and rich in enzymes. Thanks to this the liberation of folate and its dissolution in water is done already in 10 minutes.

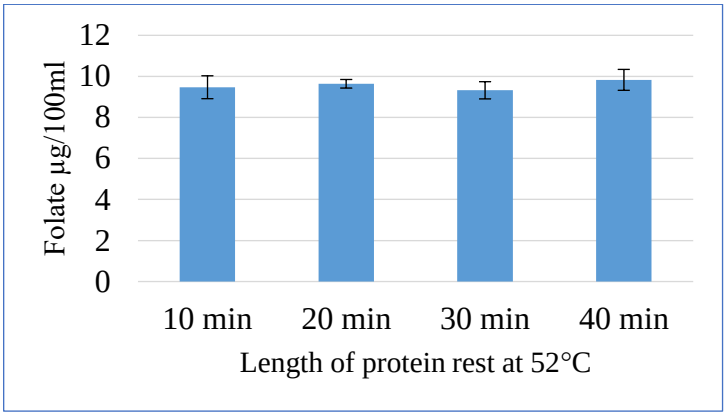


Figure 22: Evolution of folate content during an extended protein rest (mean, sd, n=3)

5.2.2 Evolution of folate content during mashing on laboratory and pilot scale

After the preliminary experiment, the evolution of folate content during laboratory and pilot scale mashing was studied. The results are presented on Figure 23. On laboratory scale after the protein rest the folate content is similar to the results of the extended protein rest since the same malt and water: malt ratio was used in both cases. Further increase was observed after the β-amylase rest at 62 °C. This can be due to the gelatinization of the starch that is between 60-63 °C in case of barley malt (PATINDOL et al. 2012). The gelatinization can help further liberation of folate which is structurally bound. At the end of the α-amylase rest there is a decrease, which can be explained with the dilution of the mash, as 100 ml water was added when reaching 72 °C to replace the evaporated water during mashing and to substitute the sparging step.

In case of pilot scale mashing an increasing tendency was observed. The difference between the detected amount of folate at the end of the protein rest is the result of different water to malt ratio at the start of mashing. In the laboratory it was 4:1 while in the pilot brewery 3:1. This different mashing-in ratio is needed because of the different conditions of filtering. In laboratory the mash

is filtered through filter paper and there is no possibility of sparging so the mash should be thinner, while in the brewery the filtration takes place in a lauter tun where sparging can be carried out resulting in the dilution of the wort, so the mash should be thicker.

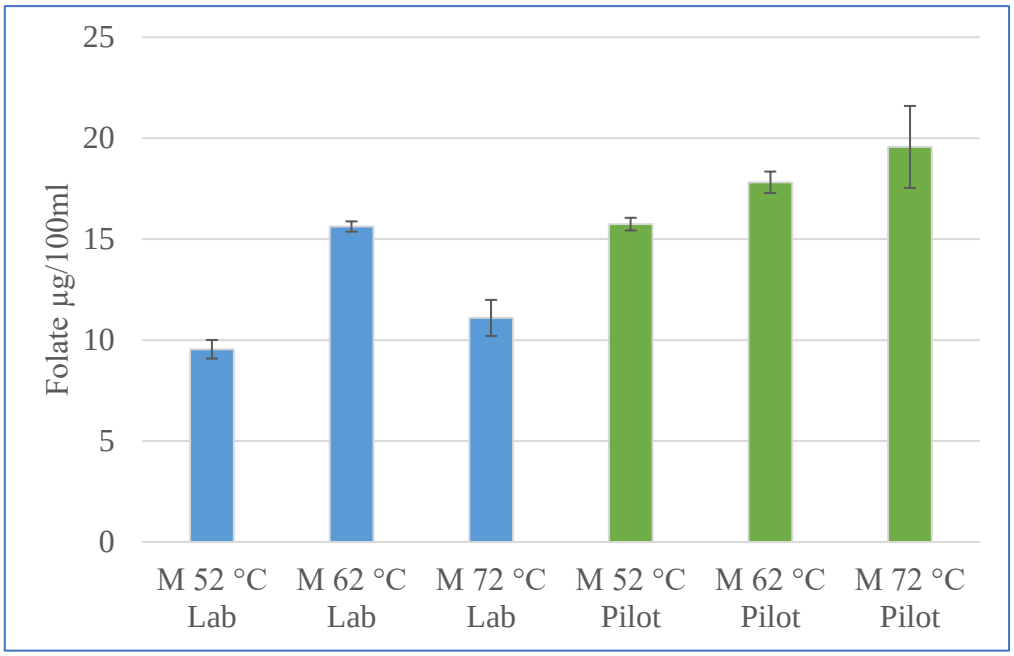


Figure 23: Evolution of folate content during laboratory and pilot scale mashing (mean, sd, n=3). M 52°C: end of 52°C enzymatic rest; M 62°C: end of 62°C enzymatic rest; M 72°C: end of 72°C enzymatic rest

5.2.3 Evolution of folate content during wort separation and hop boiling on laboratory and pilot scale

Among the brewhouse processes, wort separation and hop boiling cannot be modeled well at laboratory scale. On laboratory scale, wort separation can be carried out with the help of filter paper, while the hop boiling can be carried out on a hot plate in an Erlenmeyer flask in contrast to the specially designed equipment of a brewery.

On laboratory scale filtration through filter paper (the next technological step after mashing) does not have an influence on folate content, the folate content at the end of the 72 °C rest during mashing is the same as of the first wort. On the other hand, hop boiling seems to have a significant effect on it, but it must be taken into account that during hop boiling due to the evaporation of water, the extract content increases which affects the concentration of folate. To eliminate this, the folate content of the first wort (FW) and hopped wort (HW) was calculated reffered to 12 %m/m extract content (Table 4). The results related to the same extract content showed no difference in folate content of the first wort and hopped wort produced on laboratory

scale. It means that despite of the one hour boiling folate did not degrade, it seemed to be stable at this temperature for this period of time.

On pilot scale after the first wort separation the folate concentration decreased from 19.6 to 17.2 µg/100ml compared to the sample taken at the end of the 72 °C rest during mashing. In case of the two spargings (S1 and S2) a further decrease was observed. These results were also calculated reffered to 12 %m/m extract content, which shows that the folate content reffered to extract content of the first sparging is lower than that of the first wort while the folate content of the second sparging is higher. The sample taken before hop boiling, which contains the first wort + 2 spargings (sweet wort), shows that the two spargings together only slightly caused a decrease of the folate content of the first wort. Hop boiling was gentler in the pilot brewery which resulted in less evaporation and less extract difference between the start and end of the boiling. The folate content did not show any remarkable change during boiling on pilot scale neither in case of normal results nor in case the results calculated related to extract content. It can be stated that hop boiling which could be considered to have negative effect on folate content of beer did not decrease the concentration neither on laboratory nor on pilot scale.

Table 4: Extract content and folate content of samples taken during the brewing process (mean±sd, n=3)

	Sample name	Extract content [%m/m]	Folate content [µg/100ml]	Folate content related to 12 %m/m extract [µg/100ml]
Laboratory scale	**FW**	11.78	10.8±1.5	11.0±1.5
	HW	15.53	14.8±0.4	11.4±0.3
Pilot scale	**FW**	16.54	17.2±0.2	12.5±0.1
	S1	7.11	6.2±0.3	10.4±0.5
	S2	3.01	3.7±1.1	14.7±1.9
	SW	10.74	10.2±0.6	11.4±0.7
	HW	11.33	10.0±1.9	10.6±1.9

FW: first wort; S1-2: sparging 1-2; SW: sweet wort; HW: hopped wort

5.3 Determination of total phenolic composition and antioxidant activity from grains to malt

In this chapter the total phenolic composition and antioxidant activity results will be presented. Examination of the total phenolic composition provides an opportunity to get a picture not only of the individual target compounds identified by standards, possibly subjected to hydrolysis during sample preparation, but also of how these groups of compounds occur in their natural forms in the samples as derivatives giving a comprehensive picture of the total polyphenol profile of the samples. For the determination of antioxidant activity FRAP, TPC, CUPRAC, and ABTS assays were used.

5.3.1 Determination of total phenolic composition and antioxidant activity of malts produced from different grains

Similar to the folate results, this chapter begins with the presentation of a preliminary study where the total phenolic composition and antioxidant activity of malts made from different grains (barley, wheat, rye, oat, einkorn, emmer, spelt) were determined.

The results of antioxidant activity measurement and determination of phenols by HPLC are shown on Table 5. The results obtained in terms of Pilsner type barley malts agree the observations reported by ŠIBALIĆ et al. (2020), DVORAKOVA et al. (2008a) and LU et al. (2007) who found that the most abundant phenolic compounds of pale barley malts are catechin, epicatechin and ferulic acid. According to the results of total phenolic composition flavanol derivatives and hydroxycinnamic acid derivatives were detected in the highest concentration while hydroxybenzoic acid derivatives and flavonol derivatives were present in smaller amounts.

In terms of FRAP, TPC, CUPRAC, hydroxybenzoic acid derivatives, flavanol derivatives and total phenolic content special barley malts (caramel and coloring) show the highest values. It is due to the high temperatures these malts are treated at during the kilning or roasting process. At higher temperature MRPs and caramelization products are being formed which can contribute to antioxidant activity, furthermore the kilning step is regarded important for polyphenol solubilization (BELLMER 1978, MAILLARD 1995). Interestingly, flavonol derivatives were not detected in special malts, whereas their flavanol derivative content was much higher than the other samples. The Caramel malt had the highest hydroxycinnamic acid derivative content, while the Coloring malt showed lower values even compared to Pilsner type barley malt. It can be due to the different kilning temperatures these malts are treated at. In Caramel malt the conditions

during the liquefaction and caramelization seems to be optimal to liberate hydroxycinnamic acid derivatives but the temperature at which Coloring malt is roasted seems to be too high for this group of compounds and they are degraded.

Table 5: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) of malts produced from different grains.
Results are given in *mg ascorbic acid equivalent/100 g d.w.* for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/g d.w.* for hba-d, flava-d, hca-d, flavo-d and total phenol.

	FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
Barley malt (Pilsner)	5.2 ±0.2	13.6 ±1.1	28.1 ±1.1	111.4 ±6.5	115.7 ±2.6	430.9 ± 12.6	942.9 ±23.5	77.1 ±2.6	1566.6
Barley malt (Caramel)	16.8 ±0.7	41.8 ±3.7	76.3 ±2.7	135.8 ±4.3	553.0 ±6.4	3850.4 ±75.3	3617.7 ±138.4	n.d.	8021.1
Barley malt (Coloring)	16.2 ±0.4	43.8 ±2.4	82.1 ±5.5	151.7 ±5.2	572.8 ±8.1	9315.8 ±256.9	641.7 ±36.9	n.d.	10530.4
Wheat malt	1.4 ±0.3	9.9 ±1.0	16.1 ±0.9	99.5 ±2.7	n.d.	236.3 ±12.4	137.0 ±24.3	67.1 ±4.1	440.4
Rye malt	3.3 ±0.2	16.3 ±0.9	33.7 ±1.6	113.5 ±3.8	451.0 ±12.7	n.d.	604.8 ±15.8	32.5 ±2.4	1088.2
Oat malt	1.9 ±0.4	8.5 ±0.7	17.6 ±0.8	72.3 ±5.7	n.d.	65.5 ±3.4	65.4 ±4.2	34.9 ±4.8	165.8
Einkorn malt	1.8 ±0.1	10.8 ±1.2	15.6 ±1.3	70.6 ±3.4	215.0 ±11.3	37.7 ±5.4	57.6 ±3.5	23.6 ±3.5	333.9
Emmer malt	1.4 ±0.2	5.4 ±0.3	13.4 ±0.5	66.9 ±2.1	n.d.	49.8 ±6.8	164.1 ±11.3	32.8 ±4.1	246.7
Spelt malt	1.2 ±0.1	7.8 ±0.6	17.2 ±2.1	121.7 ±4.0	79.6 ±4.8	113.6 ±4.1	66.5 ±5.3	94.2 ±6.2	353.9

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives; n.d.:not detected

ZIELINSKI et al. (2000) reported that oat and wheat have similarly low antioxidant potential compared to barley. However, among oat, wheat and rye, the latter stands out: the results of this grain in terms of antioxidant activity in most cases exceed the values of barley malt, except for the FRAP method. It is in good correlation with its total phenolic content which is due to its high hydroxybenzoic and hydroxycinnamic acid derivative content. It even has higher hydroxybenzoic acid derivative content than Pilsner type barley malt approaching special malts with outstanding values.

The FRAP, TPC and CUPRAC values of wheat malt, oat malt, einkorn malt, emmer malt and spelt malt were the lowest. It is in agreement with the results of FOGARASI et al. (2015) who investigated the antioxidant activity of wheat, einkorn and barley malts and found that barley malt had the highest antioxidant potential according to all assays (TPC, FRAP, DPPH, ABTS).

The ABTS results showed a smaller difference between the samples than the other antioxidant activity methods.

5.3.2 Evolution of total phenolic composition and antioxidant activity during the malting process of barley

For malting the same barley varieties (Casanova and Enticel) from successive harvest (2017, 2018) years were used as in case of folate analysis. The results are presented in the same order as folate results. First, the antioxidant activity and phenolic composition of the barleys are presented, then the malts are studied and finally the effect of malting processes is investigated.

5.3.2.1 Total phenolic composition and antioxidant activity of barley

It can be seen from the results that the spring barley cultivar has higher total phenolic content in both harvest years than the winter cultivar (Table 6). In contrast, in terms of antioxidant activity, the winter cultivar shows higher values in 2018. In 2017 the spring cultivar has higher FRAP but lower TPC, CUPRAC and ABTS values than the winter one.

Table 6: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) of different barley varieties from different harvest years.
Results are given in *mg ascorbic acid equivalent/100 g d.w.* for FRAP, TPC, CUPRAC, ABTS and in *μg rutin equivalent/g d.w.* for hba-d, flava-d, hca-d, flavo-d and total phenol.

	FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
B1	1.8 ±0.1	5.3 ±0.8	14.3 ±2.1	100.4 ±6.3	53.6 ±2.4	104.1 ±2.5	143.7 ±10.6	58.2 ±6.4	359.6
B2	2.1 ±0.2	5.0 ±1.2	13.7 ±2.1	89.1 ±3.1	57.6 ±3.5	132.8 ±4.9	156.1 ±11.3	47.8 ±5.4	394.3
B3	2.7 ±0.5	4.7 ±0.9	16.8 ±0.8	92.5 ±11.3	49.6 ±5.7	164.1 ±8.2	209.3 ±4.7	35.4 ±8.1	458.4
B4	1.9 ±0.2	4.3 ±0.3	15.7 ±0.8	88.1 ±6.2	63.9 ±3.5	154.3 ±5.1	282.3 ±8.9	72.5 ±7.2	573.0

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives;
B1: Casanova winter cultivar 2017; B2: Etincel spring cultivar 2017; B3: Casanova winter cultivar 2018; B4: Etincel spring cultivar 2018

It can be concluded that winter cultivar shows higher antioxidant activity in both harvest years than spring one. On the other hand, regarding HPLC determination of total phenolic composition spring cultivars show higher total phenolic content. These differences are due to the varietal differences and also to the different harvest years, because the climatic conditions, the biotic and abiotic factors affect plant physiology and secondary metabolites such as antioxidants

(MIKKELSEN et al. 2015). These factors can induce significant differences between the antioxidant activity and phenolic composition of malting barley varieties which is supported by the observations of SHARMA et al. (2010), DVOŘÁKOVÁ et al. (2008a) and CAI et al. (2015).

No trend can be detected in the distribution of different phenolic groups in spring and winter barleys. In 2017 the spring cultivar had higher hydroxybenzoic acid derivative, flavanol derivative and hydroxycinnamic acid derivative content, and also in 2018 the hydroxybenzoic acid derivative, hydroxycinnamic acid derivative and flavonol derivative content of the spring cultivar were higher.

5.3.2.2 Total phenolic composition and antioxidant activity of barley malts

Comparing the malts produced on pilot and industrial scale I have found that both malts produced on pilot scale have higher FRAP, TPC and ABTS results, and also higher hydroxybenzoic acid derivative and flavonol content compared to the malt produced on industrial scale (Table 7). On the other hand, the total phenolic content of the pilot scale malts is less than the half of industrial scale malts which is mainly due to the differences in flavanol derivative and hydroxycinnamic acid derivative content. Maybe the circumstances during the industrial malting were better for these phenolic compounds. In case of industrial scale malts the winter cultivar has higher FRAP, CUPRAC, ABTS values but show lower result for all the phenolic compounds. In case of pilot scale malts the winter cultivar has higher TPC and ABTS results and also higher hydroxybenzoic acid content but the spring one has higher total phenolic content. The total phenolic content show correlation with the results obtained for barleys as in case of all barleys and their malts the spring cultivars had higher total phenolic content.

Table 7: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) of malts produced on different scale from different barley cultivars.
Results are given in *mg ascorbic acid equivalent/100 g d.w.* for FRAP, TPC, CUPRAC, ABTS and in *μg rutin equivalent/g d.w.* for hba-d, flava-d, hca-d, flavo-d and total phenol.

	FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
M1	8.2 ±0.7	18.6 ±2.4	47.0 ±5.1	188.7 ±8.6	142.5 ±10.2	223.3 ±4.9	149.4 ±11.7	113.4 ±11.1	628.6
M2	9.4 ±0.4	17.3 ±1.8	52.4 ±2.7	176.5 ±6.7	137.8 ±4.3	254.3 ±4.2	165.1 ±7.5	134.5 ±7.2	691.7
M3	7.2 ±0.6	13.2 ±0.8	37.3 ±3.5	148.5 ±10.5	88.8 ±8.4	402.9 ±34.2	687.9 ±22.7	53.3 ±6.2	1232.9
M4	5.2 ±0.3	13.6 ±1.1	28.1 ±2.2	111.4 ±6.7	115.7 ±10.1	430.9 ±27.4	942.9 ±27.8	77.1 ±8.8	1566.6

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives;
M1: micro scale malt of Casanova winter cultivar 2017; M2: micro scale malt of Etincel spring cultivar 2017; M3: industrial scale malt of Casanova winter cultivar 2018; M4: industrial scale malt of Etincel spring cultivar 2018

In industrial malts hydroxycinnamic acid derivatives is the most significant group of phenolic compounds contributing greatly to the total phenolic content giving more than the half of it. The less significant group of compounds is flavonol derivatives contributing about 5% to total phenolic content. These results agree with the observations reported in the literature (DVOŘÁKOVÁ et al. 2008a; CAI 2015).

In case of pilot scale malts the most significant group of compounds are flavanol derivatives which result is supported by the observations of LEITAO et al. (2012) and CARVALHO et al. (2015). On the other hand, the less significant is flavonol derivatives showing less difference in concentration than in case of industrial malts.

5.3.2.3 Changes of total phenolic composition and antioxidant activity during malting

Malts has higher values for each of the measured parameters compared to barleys, but the rate of increase of each parameter is different during malting.

The largest increase was observed in the FRAP results of the pilot scale samples (more than 4-times), also large increase was observed in the TPC and CUPRAC values (more than 3-times) (Table 8, Table 9). In case of the industrial scale samples only hydroxycinnamic acid derivative content showed more than 3-times growth (Table 10, Table 11).

Regarding the industrial scale malting of the winter cultivar almost all the parameters have a peak on the 5th day of germination, they show a continuous increase from the beginning of the malting process until the 5th day of germination, and then a decrease during the kilning process.

This decrease is the largest in its hydroxybenzoic acid derivative content which is halved to the end of the malting process compared to the 5th day of germination.

Table 8: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) during micro scale malting of Casanova winter cultivar (2017).

Results are given in *mg ascorbic acid equivalent/100 g d.w.* for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/g d.w.* for hba-d, flava-d, hca-d, flavo-d and total phenol.

	FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
B	1.8 ±0.1	5.3 ±0.8	14.3 ±2.1	100.4 ±6.3	53.6 ±2.4	104.1 ±2.5	143.7 ±10.6	58.2 ±6.4	359.6
AS	2.4 ±0.5	8.7 ±0.5	22.4 ±0.8	137.5 ±4.2	125.1 ±3.4	190.9 ±6.3	167.5 ±5.9	83.4 ±7.3	566.9
G1	3.7 ±0.3	10.1 ±0.6	28.9 ±1.6	154.3 ±1.5	183.8 ±8.1	250.3 ±4.2	176.9 ±11.8	85.1 ±5.2	696.1
G2	5.4 ±0.3	13.2 ±1.5	24.3 ±1.7	180.1 ±4.2	294.1 ±9.5	285.6 ±2.4	151.8 ±8.5	99.6 ±7.6	831.1
G3	6.3 ±0.5	17.0 ±0.4	27.7 ±0.9	178.5 ±6.8	400.2 ±6.7	325.0 ±9.3	149.7 ±4.9	103.7 ±9.1	978.6
G4	8.2 ±0.8	20.4 ±3.1	31.3 ±3.4	193.4 ±4.9	257.4 ±12.4	354.0 ±11.3	180.0 ±13.8	112.9 ±8.2	904.3
G5	8.0 ±0.2	18.5 ±1.4	28.8 ±1.2	204.2 ±8.1	301.0 ±26.8	338.9 ±16.4	193.9 ±9.1	154.1 ±14.9	987.9
M	8.2 ±0.7	18.6 ±2.4	47.0 ±5.1	188.7 ±8.6	142.5 ±10.2	223.3 ±4.9	149.4 ±11.7	113.4 ±11.1	628.6

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives;
B: barley; AS: after steeping; G1-5: germination days 1-5; M: malt

Table 9: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) during micro scale malting of Etincel spring cultivar (2017).

Results are given in *mg ascorbic acid equivalent/100 g d.w.* for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/g d.w.* for hba-d, flava-d, hca-d, flavo-d and total phenol.

	FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
B	2.1 ±0.2	5.0 ±1.2	13.7 ±2.1	89.1 ±3.1	57.6 ±3.5	132.8 ±4.9	156.1 ±11.3	47.8 ±5.4	394.3
AS	5.8 ±0.5	7.9 ±0.6	22.4 ±1.7	97.8 ±6.1	91.0 ±5.7	164.2 ±8.7	167.4 ±8.6	73.5 ±1.5	496.1
G1	6.3 ±0.4	12.5 ±0.5	27.6 ±0.9	124.6 ±4.9	112.1 ±6.1	184.1 ±7.6	173.2 ±11.9	84.7 ±7.8	554.1
G2	7.4 ±0.3	11.4 ±0.8	32.8 ±2.4	138.3 ±5.5	128.4 ±8.1	193.4 ±6.4	168.9 ±9.1	93.8 ±4.6	584.5
G3	8.2 ±0.4	15.9 ±1.8	39.1 ±1.6	168.1 ±3.8	176.5 ±4.9	232.1 ±9.3	183.5 ±18.6	121.0 ±9.5	713.1
G4	10.1 ±0.6	17.6 ±1.5	43.4 ±2.3	179.2 ±9.7	195.4 ±8.6	267.8 ±4.8	187.3 ±12.4	117.8 ±5.9	768.3
G5	10.8 ±0.3	18.3 ±2.1	50.7 ±1.5	198.5 ±5.1	217.6 ±7.9	301.7 ±8.6	194.6 ±7.8	146.9 ±5.6	860.8
M	9.4 ±0.4	17.3 ±1.8	52.4 ±2.7	176.5 ±6.7	137.8 ±4.3	254.3 ±4.2	165.1 ±7.5	134.5 ±7.2	691.7

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives;
B: barley; AS: after steeping; G1-5: germination days 1-5; M: malt

A similar trend can be seen in case of the industrial scale malting of the spring cultivar. During kilning the antioxidant activity assays show different tendencies. It is maybe due to the different sensitivity of the assays to the products being formed or degraded during kilning. According to BELLMER (1978) and LEITAO et al. (2012) the kilning step is regarded important for the solubilization of individual phenolic compounds, in contrast I observed a decrease of all the phenolic derivatives. This can be explained by the fact that although kilning has a positive role in the solubilization of individual phenolic compounds, it has a negative effect on the concentration of phenolic derivatives.

On pilot scale a similar trend is observed regarding the fact that kilning has a negative effect on the phenolic content and the hydroxybenzoic acid derivatives show the largest decrease. This group of compounds seems to be the most sensitive to heat treatment at around 85 °C. Hydroxycinnamic acid derivatives and flavonol derivatives seems to be less sensitive to the heat treatment showing a smaller decrease. Antioxidant activity values show less intensive decrease due to kilning which is in good correlation with the total phenolic content.

It has been already mentioned in the literature review that there is no consensus in the research community regarding the antioxidant activity, phenolic content and composition, and trends during malting. There are results which support my observations such as LU et al. (2007) and DVORAKOVA et al. (2008a) who also found flavanol derivatives and hydroxycinnamic acid derivatives to be the most abundant phenolic compounds in barleys and their corresponding malts. Furthermore CAI et al. (2015) identified hydroxycinnamic acids as the major phenolic acids and after malting observed a significant increase of these compounds. The increase of flavanol content and antioxidant activity is consistent with the results of CARVALHO et al. (2015). On the other hand, LEITAO et al. (2012) reported a decrease of both flavanols and antioxidant activity due to malting but as for the overall phenolic content their study showed that malting allowed a better release and extraction of phenolic compounds. The trend reported by LU et al. (2007) during the malting process is the opposite that was experienced by me: they reported a decrease of phenolic compounds and antioxidant activity during the early stages of germination, and then an increase during the later stages of germination and kilning. DVORAKOVA et al. (2008a) reported a decrease of antioxidant activity and total phenolic content due to the malting process in contrast I observed a significant increase.

Table 10: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) during industrial scale malting of Casanova winter cultivar (2018).

Results are given in *mg ascorbic acid equivalent/100 g d.w.* for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/g d.w.* for hba-d, flava-d, hca-d, flavo-d, and total phenol.

	FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
B	2.7 ±0.5	4.7 ±0.9	16.8 ±0.8	92.5 ±11.3	49.6 ±5.7	164.1 ±8.2	209.3 ±4.7	35.4 ±8.1	458.4
AS	3.4 ±0.6	6.7 ±0.4	23.4 ±1.2	118.0 ±7.4	62.9 ±6.2	208.2 ±12.3	177.4 ±3.1	48.9 ±9.2	497.4
G1	4.2 ±0.8	10.3 ±0.8	22.7 ±1.7	127.5 ±8.6	107.1 ±3.4	257.9 ±14.7	147.3 ±6.8	56.0 ±7.3	568.3
G2	4.9 ±0.4	14.2 ±0.7	22.5 ±0.9	143.8 ±12.6	181.0 ±7.9	347.3 ±16.8	300.7 ±5.0	67.2 ±4.5	896.2
G3	5.8 ±0.5	17.2 ±0.5	28.6 ±2.4	157.6 ±10.4	183.7 ±10.1	382.1 ±14.6	317.1 ±13.1	67.8 ±6.7	950.7
G4	6.4 ±0.8	17.9 ±0.6	34.8 ±1.9	164.2 ±7.2	194.7 ±8.7	439.5 ±22.4	412.6 ±12.7	70.5 ±9.8	1117.3
G5	7.0 ±0.4	18.4 ±0.9	39.1 ±2.7	183.1 ±13.8	196.8 ±9.3	542.8 ±19.5	687.3 ±14.5	68.2 ±9.7	1495.1
M	7.2 ±0.6	13.2 ±0.8	37.3 ±3.5	148.5 ±10.5	88.8 ±8.4	402.9 ±34.2	687.9 ±22.7	53.3 ±6.2	1232.9

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives;
B: barley; AS: after steeping; G1-5: germination days 1-5; M: malt

Table 11: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) during industrial scale malting of Etincel spring cultivar (2018).

Results are given in *mg ascorbic acid equivalent/100 g d.w.* for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/g d.w.* for hba-d, flava-d, hca-d, flavo-d, and total phenol.

	FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
B	1.9 ±0.2	4.3 ±0.3	15.7 ±0.8	88.1 ±6.2	63.9 ±3.5	154.3 ±5.1	282.3 ±8.9	72.5 ±7.2	573.0
AS	2.4 ±0.3	7.4 ±0.4	17.5 ±1.3	100.7 ±4.8	78.1 ±4.5	172.6 ±7.3	230.4 ±13.5	90.7 ±5.3	571.8
G1	3.1 ±0.1	8.8 ±0.8	22.6 ±0.8	114.8 ±6.3	107.9 ±6.8	190.5 ±6.5	204.2 ±22.7	85.1 ±8.1	587.7
G2	3.5 ±0.1	11.1 ±1.7	22.3 ±0.7	127.6 ±5.1	138.2 ±3.7	248.6 ±4.7	186.1 ±13.4	88.3 ±6.9	661.2
G3	3.8 ±0.2	11.9 ±0.5	25.4 ±1.5	132.1 ±8.4	251.8 ±12.8	385.7 ±7.2	418.4 ±17.3	89.2 ±7.7	1145.1
G4	4.4 ±0.2	14.5 ±0.9	28.3 ±2.7	137.5 ±6.5	299.1 ±9.1	501.1 ±12.8	689.9 ±38.9	87.7 ±11.4	1577.8
G5	5.1 ±0.4	18.3 ±0.7	28.7 ±1.3	138.7 ±4.9	355.0 ±17.6	542.2 ±26.3	833.8 ±41.2	82.9 ±9.6	1813.9
M	5.2 ±0.3	13.6 ±1.1	28.1 ±2.2	111.4 ±6.7	115.7 ±10.1	430.9 ±27.4	942.9 ±27.8	77.1 ±8.8	1566.6

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives;
B: barley; AS: after steeping; G1-5: germination days 1-5; M: malt

5.3.3 Effect of roasting on total phenolic composition and antioxidant activity

In order to study the effect of high temperature heat treatment in more depth, roasting experiments were carried out where Pilsner malt was treated at different temperatures for different time periods.

Table 12 shows that roasting at 100°C and 125°C for 20 minutes does not influence the FRAP, TPC and CUPRAC values but causes a decrease of ABTS. It is probably because at these temperatures the compounds for which ABTS is selective, are degraded.

Roasting at 150°C and 175°C induce an increase in FRAP, TPC and CUPRAC values but has the same effect on ABTS as the heat treatment at 125°C. The increase is due to the higher kilning temperature as at higher temperature MRPs and caramelization products are being formed which can contribute to antioxidant activity (MAILLARD 1995).

In terms of phenolic compounds different trends are observed of the different group of compounds. Hydroxybenzoic acid derivatives show a decrease during roasting at 100 and 125°C and then an increase when roasted at even higher temperatures. This result is in accordance with the data reported by KROL et al. (2020) who found that during the roasting of coffee beans the Gallic acid content decreased significantly when roasted at 190°C but when the temperature was increased to above 200°C the Gallic acid content started to increase resulting in a significantly higher concentration when roasted at 250°C compared to the raw coffee bean.

Flavanol derivatives and hydroxycinnamic acid derivatives show a continuous increase as the temperature is increasing, resulting in a tenfold difference between the initial malt and the roasted malt at 175°C for 20 minutes, this result is supported by KROL et al. (2020) who found that the epigallocatechin gallate content of coffee beans is increasing with increasing roasting temperature.

On the other hand, flavonol derivatives are decreasing along with the increasing temperature. The total phenolic content shows a continuous growth primarily due to the change of flavanol derivatives and hydroxycinnamic acid derivatives.

Table 12: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) during roasting at different temperature for 20 minutes.

Results are given in *mg ascorbic acid equivalent/100 g d.w*. for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/g d.w.* for hba-d, flava-d, hca-d, flavo-d, and total phenol.

	FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
Malt	8.2 ±1.1	18.6 ±0.5	47.0 ±3.2	188.7 ±4.8	142.5 ±8.4	223.3 ±13.5	149.4 ±8.6	113.4 ±8.7	628.6
100°C/20 min.	8.4 ±0.8	17.6 ±0.8	45.8 ±1.2	154 .6 ±9.2	107.8 ±6.9	277.2 ±9.2	264.2 ±16.3	107.3 ±9.3	756.4
125°C/20 min.	8.1 ±1.3	15.5 ±0.7	45.0 ±4.1	117.4 ±7.5	105.2 ±10.1	241.4 ±12.1	496.5 ±17.5	94.5 ±9.4	937.6
150°C/20 min.	11.4 ±1.1	19.5 ±0.7	58.4 ±2.5	114.8 ±9.8	148.9 ±8.7	460.4 ±18.7	1147.8 ±72.8	80.7 ±8.6	1837.8
175°C/20 min.	13.9 ±0.7	26.3 ±0.9	66.2 ±5.8	123.9 ±8.3	200.3 ±11.2	1993.2 ±63.5	1447.8 ±67.4	59.5 ±7.1	3700.7

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives

Roasting at 200°C (Table 13) induced an increase also in FRAP, TPC and CUPRAC values compared to the initial malt but this increase shows a peak when roasted for 15 minutes at 200°C then a slight decrease was observed when roasting for 20 minutes at 200°C. In case of ABTS a continuous decrease can be observed with increasing time. SAMARAS et al. (2005) have reported that some thermally induced compounds possessing antioxidant activity may degrade on prolonged heating, they have observed a loss of compounds after 10 minutes at 220 ºC. It seems that the duration of the treatment at the final kilning temperature in case of coloring malts is very important from the aspect of antioxidant activity because different time periods at temperatures around 200°C affect antioxidant activity differently.

In terms of phenolic compounds, the hydroxycinnamic acid derivative content showed a huge increase after 5 minutes at 200°C but then no considerable change was observed. Hydroxybenzoic acid derivatives and flavanol derivatives show a continuous increase with increasing time. Flavanol derivative content is continuously increasing from the gentlest roasting at 100°C for 20 minutes until the most intensive roasting level at 200°C for 20 minutes. This suggests a continuous liberation of flavanol derivatives and a good thermal stability of these compounds. An interesting trend is observed in case of flavonol derivatives, the concentration decreases when roasting for 5-10 minutes and then increases when roasting for 15-20 minutes at 200°C resulting in a similar concentration when roasted for 20 minutes at 200°C as of the initial malt. The total phenolic concentration shows continuous growth with increasing time at 200°C resulting in a more than tenfold growth which is considerably influenced by the flavanol derivative content.

Table 13: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) during roasting at 200 °C for different time periods.

Results are given in *mg ascorbic acid equivalent/100 g d.w.* for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/g d.w.* for hba-d, flava-d, hca-d, flavo-d, and total phenol.

Effect of roasting on total phenolic composition and antioxidant activity									
	FRAP	**TPC**	**CUPRAC**	**ABTS**	**hba-d**	**flava-d**	**hca-d**	**flavo-d**	**Total phenol**
Malt	8.2 ±0.2	18.6 ±2.4	47.0 ±3.5	188.7 ±10.7	142.5 ±8.7	223.3 ±12.5	149.4 ±10.1	113.4 ±8.4	628.6
200°C/5 min.	12.7 ±1.2	36.5 ±2.7	67.6 ±4.8	123.3 ±10.4	200.1 ±12.8	1240.2 ±69.4	1276.0 ±56.9	41.9 ±6.2	2758.2
200°C/10 min.	13.2 ±1.3	30.3 ±2.5	65.4 ±4.5	110.6 ±8.6	257.3 ±14.6	4418.7 ±158.2	1339.6 ±81.3	32.6 ±4.5	6048.3
200°C/15 min.	13.8 ±1.1	33.3 ±3.1	70.5 ±6.2	110.8 ±9.4	290.7 ±11.5	5734.9 ±199.3	1317.6 ±67.8	68.8 ±6.9	7412.0
200°C/20 min.	13.2 ±0.9	29.9 ±1.7	68.8 ±3.1	100.2 ±6.8	301.0 ±18.3	6237.7 ±175.7	1249.2 ±57.6	106.1 ±7.8	7894.0

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives

Large differences were observed between the effect of kilning at the end of malting and the effect of roasting experiments on antioxidant activity and phenolic content. There are several factors which differ between the kilning step at the end of the malting process and the roasting experiments. The first is the moisture content. While during the malting process the green malt has approximately 45% moisture content before kilning, in case of the roasting experiments a Pilsner type malt was roasted which has a moisture content of ~4%. The difference in moisture content greatly influences the chemical reactions taking place, e.g., due to the effect of oxidizing enzymes these compounds degrade more easily at higher moisture contents.

At the end of the malting process, the main purpose of kilning is the structural and chemical stabilization of the malt, to preserve the activity of the enzymes and to increase the shelf life of the malt. This process stabilizes the malt-building compounds and creates a structure that is least sensitive to external impacts. On the other hand, the purpose of roasting is the formation of aroma and coloring compounds to give the final beer a special character.

5.4 Total phenolic composition and antioxidant activity of hops

In addition to malt, hop is the other raw material which can contribute to the antioxidant activity and phenolic content of beer. Therefore, different hop varieties with different alpha acid content were examined. The total phenolic composition, antioxidant activity and α-acid content of the samples are presented in Table 14. The investigated hops show a very diverse antioxidant activity and phenolic composition. It is due to the dependence of the antioxidant potential and composition on both the variety and the growing locality (OLŠOVSKÁ et al. 2013).

Hops have much higher antioxidant activity and total phenolic content than the investigated barley malts, regarding phenolic compounds in some cases an order of magnitude higher. This result is consistent with the data reported by ALMAGUER et al. (2014) who found the concentration of polyphenols in barley malt to be 50-100 mg/100g while in hops up to 4g/100g.

The most dominant phenolic components are flavonol derivatives, but some samples have high flavanol derivative and hydroxycinnamic acid derivative content too. In contrast ŠIBALIĆ et al. (2020) found flavanol derivatives to be the most abundant phenolic compounds detected in hop extracts but also found high concentrations of flavonol derivatives. The differences can be because of the different methods and qualitative or quantitative determination of the phenolic compounds, furthermore environmental factors and the variety also influence the phenolic composition. There are samples for which no hydroxybenzoic acid derivative or flavanol derivative content was detected.

As it was mentioned in the literature review, information reported on the relationship between alpha acid content and polyphenol content, or antioxidant activity is not consistent in the scientific literature. Therefore, Pearson's correlation test was performed, the results of which can be seen on Table 15. According to the results only TPC shows a positive significant correlation with the alpha acid content, there is no significant correlation between the other antioxidant assays or phenolic compounds and alpha acid content. The positive correlation of TPC is consistent with the data published by LIU et al. (2007) who reported that the higher the bitter acids content is, the stronger the antioxidant activity of hops is, nevertheless, the opposite was observed for the other antioxidant assays.

Table 14: Antioxidant activity (mean±sd, n=3), total phenolic composition (mean±sd, n=2) and alpha-acid content of hops.

Results are given in *mg ascorbic acid equivalent/100 g d.w*. for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/g d.w*. for hba-d, flava-d, hca-d, flavo-d, and total phenol.

Total phenolic composition and antioxidant activity of hops										
	α-acid	**FRAP**	**TPC**	**CUPRAC**	**ABTS**	**hba-d**	**flava-d**	**hca-d**	**flavo-d**	**Total phenol**
Chinook	11.6	16.8 ±3.5	95.1 ±5.6	87.7 ±6.3	207.5 ±5.6	512.1 ±12.5	3133.3 ±125.6	914.4 ± 14.3	5681.1 ±265.4	10240.9
Cashmere	7.1	18.2 ±1.5	90.5 ±8.1	81.1 ±2.8	225.0 ±8.4	459.5 ±17.8	2279.2 ±147.9	1900.9 ±59.8	5658.3 ±185.1	10297.9
Ctz	15.7	18.8 ±2.4	125.8 ±8.6	89.7 ±5.7	273.8 ±9.1	444.4 ±15.6	2207.3 ±175.6	991.1 ±70.4	5211.2 ±173.8	8854.1
Comet	6.4	14.6 ±1.6	77.4 ±7.2	78.6 ±5.4	158.1 ±5.2	178.4 ±17.8	958.7 ±56.7	1384.5 ±85.6	1982.7 ±101.0	4504.3
Tahoma	6.9	23.1 ±1.8	115.9 ±9.3	99.7 ±4.6	273.8 ±5.6	344.4 ±16.8	396.0 ±21.4	1790.2 ±69.2	4712.4 ±149.3	7243.0
Denali	15.4	17.3 ±2.1	110.2 ±5.4	81.6 ±4.1	290.7 ±6.4	361.5 ±14.8	1371.9 ±59.9	3902.7 ±231.4	7472.9 ±217.5	13109.0
Sorachi Ace	12.9	11.4 ±1.2	74.4 ±4.7	79.7 ±6.7	122.2 ±4.8	237.9 ±9.9	751.1 ±16.7	1263.4 ±47.5	5927.3 ±201.9	8179.7
Crystal	4.9	22.8 ±1.9	102.8 ±2.6	102.6 ±5.3	251.2 ±8.7	268.7 ±12.8	423.4 ±14.1	1646.8 ±122.3	5858.4 ±187.9	8197.3
Centennial	8.9	15.1 ±0.9	94.0 ±8.1	76.7 ±4.1	229.6 ±8.9	259.3 ±11.0	1362.7 ±32.8	457.4 ±25.9	4458.9 ±156.3	6538.3
Mt. Hood	5.2	19.8 ±2.1	97.6 ±4.3	94.5 ±5.7	214.3 ±8.4	328.1 ±10.7	442.4 ±11.1	1894.1 ±85.4	7557.8 ±254.1	10222.4
Pekko	16.8	22.5 ±0.8	140.3 ±6.1	101.4 ±4.8	225.2 ±6.5	123.9 ±11.8	346.5 ±12.0	1323.8 ±93.2	4738.4 ±189.7	6532.6
Nugget	15.5	16.1 ±1.3	112.0 ±3.5	89.6 ±5.9	254.6 ±11.5	n.d.	218.0 ±8.9	1785.7 ±85.2	8383.2 ±234.7	10386.9
Columbus	17.4	18.8 ±0.9	116.9 ±5.7	91.3 ±6.1	274.6 ±4.9	n.d.	1282.1 ±62.4	2298.5 ±99.6	5274.6 ±215.9	8855.2
Belma	10.7	15.6 ±1.8	90.9 ±4.3	95.3 ±5.0	167.7 ±12.5	n.d.	n.d.	1736.2 ±73.5	7221.7 ±197.9	8957.9
Azacca	14.4	19.5 ±1.2	119.7 ±6.2	95.2 ±4.7	250.0 ±11.9	n.d.	n.d.	983.4 ±56.4	2843.0 ±100.8	3826.4
Willamette	4.8	24.0 ±1.0	103.5 ±5.4	103.9 ±3.8	268.7 ±10.0	n.d.	n.d.	2434.9 ±112.8	9211.6 ±268.1	11646.5

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives

Between the antioxidant activity assays positive significant correlation was observed except for CUPRAC and DPPH which do not show a significant correlation. Neither of the antioxidant activity assays show positive significant correlation with any of the phenolic compounds which is consistent with the results reported by OLADOKUN et al. (2016), regarding CUPRAC assay negative significant correlation was found with flavanol derivatives. Phenolic compounds show positive significant correlation with each other in some cases e.g., hydroxybenzoic acid derivatives with flavanol derivatives, hydroxycinnamic acid derivatives with flavonol derivatives and total phenols, and flavonol derivatives with total phenols. Based on my results, no clear

conclusion can be drawn about the correlations between alpha acid content, antioxidant activity and phenolic composition of hops, except that there are large differences between the samples which can be influenced by many factors.

Table 15: Pearson's correlation matrix of antioxidant activity, total phenolic composition, and alpha-acid content of hops. Numbers in bold show significant results ($p<0.05$)

		Pearson r									
		α-acid	**FRAP**	**TPC**	**CUPRAC**	**ABTS**	**hba-d**	**flava-d**	**hca-d**	**flavo-d**	**Total phenol**
Pearson p	**α-acid**	/	-0.250	**0.536**	-0.133	0.184	-0.210	0.144	0.039	-0.076	-0.007
	FRAP	0.350	/	**0.638**	**0.839**	**0.657**	-0.056	-0.279	0.214	0.149	0.074
	TPC	**0.032**	**0.008**	/	**0.560**	**0.720**	-0.161	-0.138	0.090	-0.007	-0.041
	CUPRAC	0.624	**< 0.0001**	**0.024**	/	0.377	-0.352	**-0.539**	0.100	0.279	0.018
	ABTS	0.496	**0.006**	**0.002**	0.151	/	0.020	0.032	0.410	0.218	0.313
	hba-d	0.435	0.836	0.552	0.181	0.943	/	**0.759**	-0.078	-0.108	0.252
	flava-d	0.594	0.295	0.610	**0.031**	0.906	**0.001**	/	-0.148	-0.174	0.252
	hca-d	0.885	0.426	0.740	0.713	0.115	0.774	0.583	/	**0.538**	**0.674**
	flavo-d	0.780	0.583	0.978	0.295	0.418	0.690	0.520	**0.031**	/	**0.870**
	Total phenol	0.980	0.786	0.881	0.949	0.238	0.346	0.346	**0.004**	**< 0.0001**	/

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives.

5.5 Changes of total phenolic composition and antioxidant activity during brewing

Similar to the folate results, after studying the raw materials, first the mashing on laboratory and pilot scale was compared, then the wort separation and hop boiling and finally the effect of fermentation was investigated on total phenolic composition and antioxidant activity. For these experiments in addition to pale worts, dark worts and beers were also prepared using special caramel and coloring malts in order to investigate the effect of the higher antioxidant activity and phenolic content of these malts on the samples taken during the brewing process.

5.5.1 Effect of mashing on phenolic composition and antioxidant activity

The antioxidant activity and phenolic composition of the samples are shown in Table 16. Until the end of the first enzymatic rest (52°C), regardless of the production scale, water-soluble antioxidants already go into solution, furthermore these temperatures are optimal for protease and β-glucanase enzymes of barley malt which can release antioxidants bound to cell walls, polysaccharides, or proteins. It is consistent with the result of SCHWARZ et al. (2012), who have reported the same temperature as ideal one for polyphenol release from malt, and with the results of ZHAO et al. (2012) who have found that antioxidant activity increased the most intensive during the early stage of mashing. Hereinafter all the results increased between the end

of the 52°C and 62°C rests. This is due to the enzymatic activity which liberates compounds with antioxidant activity including phenolic compounds.

Table 16: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) during mashing on laboratory scale.

Results are given *in mg ascorbic acid equivalent/100 cm^3* for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/cm^3* for hba-d, flava-d, hca-d, flavo-d, and total phenol.

		FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
Lab. pale mashing	**52 °C**	5.0 ±0.3	46.7 ±1.1	32.1 ±2.7	n.d.	295.7 ±8.6	77.6 ±4.6	6.3 ±1.0	46.1 ±3.6	425.7
	62 °C	5.1 ±0.4	58.3 ±1.3	34.9 ±2.5	11.3 ±0.8	545.2 ±16.8	116.4 ±5.2	6.9 ±0.5	122.2 ±8.4	790.7
	72 °C	6.1 ±0.3	50.3 ±2.2	35.8 ±1.9	25.4 ±3.2	488.5 ±22.5	111.5 ±3.7	6.3 ±0.7	120.5 ±3.5	726.7
Lab. dark mashing	**52 °C**	14.6 ±0.5	114.4 ±4.5	92.7 ±2.7	n.d.	568.1 ±28.1	248.7 ±29.4	164.4 ±7.9	20.6 ±3.2	1001.8
	62 °C	20.0 ±1.0	129.2 ±4.2	105.8 ±5.1	55.9 ±2.9	854.3 ±37.3	436.7 ±17.8	193.1 ±12.5	41.8 ±5.6	1525.9
	72 °C	22.3 ±0.8	119.6 ±5.3	108.0 ±5.8	56.5 ±4.1	744.9 ±28.4	405.3 ±27.8	152.2 ±11.7	36.2 ±2.4	1338.7

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives;
52 °C: end of 52 °C rest; 62 °C: end of 62 °C rest; 72 °C: end of 72 °C rest; n.d.: not detected

When reaching 72°C FRAP, CUPRAC and ABTS show further increase but TPC decreases in case of laboratory scale mashing of both the pale and dark worts. These results are interesting because the addition of 100 ml water to the mash on laboratory scale when reaching 72°C (to substitute the sparging during wort separation), decreased the TPC value, while all the other antioxidant assay increased. The increase is maybe due to the further liberation of compounds induced by enzymatic activity and higher temperature. On the other hand, the concentration of all the phenolic compounds decreased due to the dilution do not show correlation with the antioxidant activity assays.

Regarding pilot scale brewing of the pale beer the increase of antioxidant activity and phenolic content stops and stagnates or even decreases slightly after the 62 °C rest until the end of the 72 °C rest (Table 17).

Table 17: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) during mashing on pilot scale.

Results are given *in mg ascorbic acid equivalent/100 cm³* for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/cm³* for hba-d, flava-d, hca-d, flavo-d, and total phenol.

		FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
Pilot pale mashing	**52 °C**	10.2 ±0.4	74.2 ±2.8	71.5 ±3.1	1.5 ±0.3	788.5 ±32.6	370.2 ±24.8	62.8 ±3.7	37.7 ±5.7	1259.2
	62 °C	13.1 ±0.4	86.2 ±3.4	79.1 ±2.5	36.8 ±2.1	877.6 ±31.7	381.6 ±21.6	67.0 ±5.4	45.1 ±4.1	1371.3
	72 °C	13.2 ±0.3	86.4 ±3.9	78.0 ±3.4	91.9 ±3.6	875.9 ±26.4	383.9 ±17.9	63.9 ±4.2	43.5 ±3.8	1367.2
Pilot dark mashing	**52 °C**	18.7 ±2.1	109.5 ±7.3	97.7 ±3.5	26.7 ±3.2	395.6 ±12.6	115.2 ±10.2	60.2 ±4.9	16.4 ±3.2	587.4
	62 °C	22.8 ±1.8	119.2 ±4.9	104.5 ±4.2	70.1 ±2.3	531.5 ±17.8	210.6 ±12.7	68.5 ±5.7	25.3 ±2.7	835.9
	72 °C	29.7 ±3.5	135.0 ±8.6	114.8 ±6.1	115.6 ±5.8	1281.7 ±47.4	556.2 ±27.6	187.7 ±10.8	34.7 ±2.9	2060.3

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives;
52 °C: end of 52 °C rest; 62 °C: end of 62 °C rest; 72 °C: end of 72 °C rest; n.d.: not detected

This is an interesting result compared to laboratory scale mashing, where with the dilution a similar tendency was observed. It can be explained by the observations of ZHAO et al. (2015) who reported that above 65 °C no enzyme activity related to release of phenolic compounds was detected. In case of the pilot scale dark wort all the results show a continuous increase especially when the temperature is raised to 72 °C. There is a considerable increase of hydroxybenzoic acid derivatives, flavanol derivatives and hydroxycinnamic acid derivatives between the 62 °C and 72 °C rest. This is due to the chocolate malt which was added when reaching 72 °C because as it was reported before, these types of special malts contain high concentration of these phenolic compounds but no or very low concentration of flavonol derivatives.

The results given by ABTS assay at the early stages of the brewing process both under laboratory circumstances and in the pilot brewery were unexpected. ABTS does not show any relevant antioxidant activity at the beginning of mashing but afterwards increases radically. In case of this assay the above elaborated theory about the importance of the protease, β-glucanase rest at the beginning of mashing is inappropriate. Compounds measured by this method can be either soluble at higher temperature or they are formed at higher temperature.

5.5.2 Effect of wort separation and hop boiling on phenolic composition and antioxidant activity

In order to see the effect of first wort separation and make the comparison easier the results at the end of mashing and the results of the first worts are presented in Table 18.

Between the FRAP, TPC, and CUPRAC results of the 72 °C and first wort samples there is no remarkable change observed, a slight increase was observed in some samples and a slight decrease in others. On the other hand, ABTS showed considerable changes, it increased during the first wort separation of both laboratory scale samples but decreased on pilot scale.

The change of ABTS values during wort separation is interesting as there is no concentration change during wort separation. There are two differences between laboratory scale and pilot scale first wort samples which could explain the opposing trends. First, the laboratory scale samples were filtered immediately at the end of the 72 °C rest while on pilot scale the temperature was raised to 78 °C at the end of mashing prior to lautering. The second difference is that in the laboratory filter papers were used for wort separation while in the pilot brewery the wort was separated in a lauter tun. The differences may be explained by heating the mash to 78 °C and performing first wort separation in lauter tun at this higher temperature. It most probably has negative effect on compounds that are detected with the ABTS method.

The concentration of phenolic compounds shows a slight decrease on both laboratory and pilot scale, which may be due to the fact that some of the phenolic compounds which are in solution during mashing are bound on the filter layer during filtration. These results do not agree with the data reported by PASCOE et al. (2003) who observed an increase of both antioxidant activity and phenolic compounds after wort separation.

Table 18: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) during first wort separation on laboratory and pilot scale.

Results are given in *mg ascorbic acid equivalent/100 cm*^{3} for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/cm*^{3} for hba-d, flava-d, hca-d, flavo-d, and total phenol.

		FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
Lab. pale mashing	**72 °C**	6.1 ±0.7	50.3 ±3.5	35.8 ±2.7	**25.4** ±2.7	488.5 ±12.7	111.5 ±8.2	6.3 ±0.8	120.5 ±8.1	726.7
	FW	5.5 ±0.4	49.5 ±4.2	35.0 ±1.5	**79.4** ±2.9	466.6 ±19.2	110.7 ±5.9	6.1 ±0.5	111.6 ±11.0	695.0
Lab. dark mashing	**72 °C**	22.3 ±0.9	119.6 ±4.7	108.0 ±5.8	**56.5** ±3.5	744.9 ±24.7	405.3 ±17.8	152.2 ±9.5	36.2 ±5.1	1338.7
	FW	21.9 ±1.6	125.6 ±2.8	109.5 ±4.3	**114.1** ±8.2	663.6 ±22.8	392.1 ±24.5	138.7 ±4.7	32.5 ±3.4	1226.9
Pilot pale mashing	**72 °C**	13.2 ±0.9	86.4 ±5.7	78.0 ±4.3	**91.9** ±4.3	875.9 ±28.9	383.9 ±14.7	63.9 ±7.1	43.5 ±3.7	1367.2
	FW	15.7 ±1.1	91.4 ±3.6	87.0 ±3.6	**47.1** ±3.7	839.7 ±19.6	364.6 ±9.6	47.5 ±3.2	42.1 ±2.5	1293.9
Pilot dark mashing	**72 °C**	29.7 ±1.2	135.0 ±7.4	114.8 ±6.7	**115.6** ±7.6	1281.7 ±75.3	556.2 ±11.7	187.7 ±11.8	34.7 ±4.2	2060.3
	FW	30.3 ±2.7	137.9 ±6.1	115.5 ±8.5	**89.4** ±5.7	1123.4 ±64.1	416.5 ±18.1	201.2 ±17.6	35.4 ±2.7	1776.5

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives

72 °C: end of 72 °C rest; FW: first wort

Effect of wort boiling is evaluated by analyzing data of first worts, spargings, sweet worts (first wort+spargings) and hopped worts. In order to eliminate the differences in extract content and its effect on antioxidant activity and phenolic content, the results were calculated reffered to 12 %m/m extract content and are presented in Table 19.

The pilot scale pale first wort has higher antioxidant activity and phenolic content compared to the laboratory scale pale first wort, except for ABTS and flavonol derivatives. Regarding dark worts, there is no clear difference in favor of any scales, the largest differences are in ABTS and hydroxybenzoic acid derivatives.

On pilot scale after first wort separation two spargings were carried out. As it is shown in Table 19 the extract content of the spargings is much lower than of the first wort. Despite of this the antioxidant activity of the pale wort related to extract content is higher for the two spargings than the first wort. Regarding phenolic compounds the values of the first sparging is similar to the first wort but the second sparging contain higher concentration of phenolic compounds than the first wort. With respect to the dark wort similarly the second sparging has higher results. It means that related to extract content more phenolic compounds are dissolved from the spent grain during sparging than in case of first wort.

The biggest increase was observed in case of ABTS values of the second sparging of both the pale and dark worts. This can be related to the previous observation, where compared to the end

of mashing, the ABTS decreased significantly in the first wort on pilot scale. This decrease is probably due to the fact that the compounds to which ABTS is selective are bound to the filter layer (namely spent grain) during first wort separation and could be dissolved therefrom by the hot water used during sparging, resulting in higher ABTS values related to extract content. Due to the high ABTS values of the second sparging the ABTS values in the sweet wort multiplied compared to the first worts.

During hop boiling antioxidant activity changed in the same way for both scale pale worts and for both scale dark worts. Regarding pale worts antioxidant activity increased but for dark worts only FRAP and TPC increased, CUPRAC and ABTS decreased. This shows that production scale is not relevant for antioxidant activity during hop boiling.

Table 19: Antioxidant activity (mean±sd, n=3), total phenolic composition (mean±sd, n=2) and extract content during wort separation and hop boiling on laboratory and pilot scale.
All the antioxidant and phenolic results are calculated reffered to 12 %m/m extract content.
Results are given in *mg ascorbic acid equivalent/100 cm*^{3} for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/cm*^{3} for hba-d, flava-d, hca-d, flavo-d, and total phenol.

		Extract content (%m/m)	FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
Lab. pale	**FW**	11.78	5.6 ±1.0	50.4 ±4.2	35.7 ±3.1	80.9 ±2.7	475.4 ±16.5	112.8 ±6.2	6.2 ±0.4	113.7 ±9.3	708.0
	HW	15.53	12.2 ±1.1	64. 2 ±3.7	65.0 ±2.7	96.9 ±4.1	457.3 ±9.4	98.0 ±4.8	n.d.	101.1 ±5.7	656.4
Pilot pale	**FW**	16.54	11.4 ±0.8	66.3 ±2.8	63.1 ±4.5	34.2 ±3.8	609.2 ±19.9	264.5 ±7.1	34.5 ±2.3	30.5 ±2.8	938.7
	S 1	7.11	12.1 ±0.5	73.6 ±4.5	74.0 ±2.1	71.1 ±2.7	588.2 ±17.5	254.3 ±15.3	41.0 ±3.1	28.4 ±3.6	911.9
	S 2	3.01	12.6 ±1.1	77.7 ±3.7	84.3 ±5.0	307.6 ±4.6	709.2 ±32.4	283.5 ±7.9	46.6 ±3.0	32.3 ±2.7	1071.6
	SW	10.74	12.2 ±1.0	70.6 ±2.5	66.1 ±3.7	73.6 ±6.8	604.4 ±20.0	254.9 ±15.6	51.3 ±2.7	28.4 ±3.5	938.9
	HW	11.33	14.2 ±0.8	74.8 ±4.0	73.5 ±1.2	73.0 ±4.2	579.1 ±14.3	256.1 ±12.4	52.9 ±5.8	29.0 ±3.2	917.1
Lab. dark	**FW**	16.38	16.1 ±1.2	92.0 ±3.5	80.2 ±3.4	83.6 ±3.1	486.1 ±18.7	287.3 ±10.1	101.6 ±5.2	23.8 ±4.3	898.8
	HW	17.20	18.3 ±1.1	92.8 ±6.1	77.7 ±3.2	81.0 ±4.7	544.2 ±7.2	316.4 ±9.7	103.5 ±8.3	24.7 ±2.1	988.8
Pilot dark	**FW**	18.74	19.4 ±1.7	88.3 ±1.4	73.9 ±2.8	57.3 ±3.2	719.4 ±11.5	266.7 ±13.8	128.8 ±7.1	22.7 ±1.3	1114.9
	S 1	14.05	18.0 ±1.5	85.1 ±2.9	84.5 ±4.5	48.6 ±2.7	724.5 ±17.3	294.8 ±17.2	121.7 ±6.4	24.3 ±1.7	1141.1
	S 2	8.02	19.2 ±1.4	96.9 ±3.8	100.6 ±1.2	124.6 ±1.9	782.8 ±12.5	327.4 ±12.4	122.5 ±7.2	21.5 ±1.9	1232.8
	SW	13.25	20.3 ±1.5	91.0 ±2.7	88.2 ±3.6	115.7 ±3.5	762.3 ±14.8	318.3 ±20.0	133.3 ±8.5	26.2 ±1.8	1213.9
	HW	14.71	21.2 ±1.3	94.8 ±4.7	87.4 ±4.5	102.9 ±6.2	839.0 ±27.6	374.9 ±18.7	141.8 ±4.3	25.6 ±1.1	1355.7

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives;
FW: first wort; S1-2: sparging 1-2; SW: sweet wort; HW: hopped wort; n.d.: not detected

On the other hand, in case of the laboratory scale pale wort, where antioxidant activity showed the biggest increase during hop boiling, the phenolic content decreased. Similar results were obtained for pilot scale pale wort, but the decrease of phenolic compounds is smaller. The increase of the antioxidant values is due to the fact that during boiling Maillard reaction products are developed which contribute to antioxidant activity (PASCOE et al. 2003). On the other hand, the loss of phenolic compounds is due to the degradation of some phenolic compounds caused by the heat impact and the complex formation and precipitation of phenolic compounds with proteins which will be separated with the hot trub (KÜHBECK et al. 2006, ŠIBALIĆ et al. 2020). The greater decrease in phenolic content of the laboratory scale pale wort is due to the more intensive boiling, which is well observed from the extract content differences between the beginning and the end of the boiling (laboratory scale extract difference: 3.75 %m/m; pilot scale extract difference: 0.59 %m/m). This difference is due to the poorer controllability of boiling in the laboratory.

While the antioxidant activity of dark worts increases in some methods and decreases in others, the phenolic content shows a clear increase during hop boiling. The above stated reason that more intensive boiling results in more intensive loss of phenolic compounds is not correct in case of dark worts because the extract difference of the pilot scale dark wort between the beginning and end of the hop boiling is 1.46 %m/m, which is more than two times of the pilot scale pale wort where decrease of phenolic content was observed. It is due to the defensing effect of melanoidins and reductons originating from the special malt which can prevent the oxidation of phenolic compounds. Furthermore, dark worts have a much different phenolic composition which behave differently during hop boiling than in case of the pale worts (ZHAO et al. 2012).

Dark worts have higher antioxidant activity, higher concentration of hydroxybenzoic acid derivatives, flavanol derivatives, hydroxycinnamic acid derivatives and total phenolic content but lower flavonol derivative content. These results are consistent with the trends observed during the roasting experiment that special malts, which are treated at higher temperature has higher antioxidant activity, and also have higher hydroxybenzoic acid derivative, flavanol derivative, hydroxycinnamic acid derivative, total phenolic content but lower flavonol derivative content.

5.5.3 Effect of fermentation on antioxidant activity and phenolic composition

The fermentation of the worts was carried out only on pilot scale in cylinder conical tanks as the yeast separation, lagering under pressure and regular sampling would not have been feasible

properly on laboratory scale. On laboratory scale the experiment ended after cooling the hopped wort and separation of the hot trub.

During the 5 days of main fermentation and 3 weeks of lagering the antioxidant activity of the dark wort slowly decreases, while in case of the pale wort only FRAP and CUPRAC shows a decreasing tendency (Table 20). Regarding phenolic compounds only flavanol derivatives of both worts and the total phenolic content of the dark wort show a clear decreasing tendency, correlating well with the antioxidant results. It can be the result of the pH drop during fermentation, due to which a number of colloidally dissolved bitter substances and polyphenols reach their isoelectric point and then precipitate.

Table 20: Antioxidant activity (mean±sd, n=3) and total phenolic composition (mean±sd, n=2) during fermentation on pilot scale.

Results are given in *mg ascorbic acid equivalent/100 cm^3* for FRAP, TPC, CUPRAC, ABTS and in *µg rutin equivalent/cm^3* for hba-d, flava-d, hca-d, flavo-d, and total phenol.

		FRAP	TPC	CUPRAC	ABTS	hba-d	flava-d	hca-d	flavo-d	Total phenol
Pilot pale	MF1	13.5 ±0.8	56.4 ±1.3	67.5 ±2.1	69.0 ±2.2	321.2 ±9.6	183.4 ±3.7	45.4 ±2.7	23.5 ±3.0	573.5
	MF2	12.6 ±0.6	52.8 ±2.5	65.4 ±2.2	61.6 ±4.3	331.3 ±12.7	150.6 ±8.2	61.1 ±3.4	28.9 ±1.7	571.9
	MF3	13.6 ±0.9	58.1 ±3.1	68.6 ±1.2	67.1 ±3.8	340.7 ±10.0	141.9 ±5.4	59.4 ±6.2	28.0 ±2.8	570.0
	MF4	13.4 ±1.1	58.1 ±2.2	66.7 ±3.0	63.4 ±1.7	336.5 ±6.8	151.2 ±3.9	65.1 ±2.4	30.0 ±3.1	582.8
	MF5	13.0 ±0.3	56.8 ±1.7	64.2 ±1.8	67.3 ±2.6	342.6 ±13.5	147.1 ±9.8	63.8 ±3.9	29.5 ±3.1	583.0
	L1	13.5 ±0.4	56.2 ±3.0	64.9 ±2.1	68.9 ±4.2	333.7 ±15.7	139.4 ±4.1	56.3 ±3.7	24.2 ±2.4	553.6
	L2	12.7 ±0.6	59.0 ±1.2	64.1 ±2.3	73.3 ±3.7	352.0 ±9.3	154.2 ±4.7	66.6 ±4.8	30.4 ±1.5	603.2
	L3	12.8 ±0.3	57.8 ±1.8	64.0 ±1.0	70.5 ±2.9	345.4 ±8.4	145.5 ±3.2	65.3 ±4.5	29.8 ±2.9	586.0
Pilot dark	MF1	26.5 ±0.8	109.4 ±2.9	106.7 ±2.7	126.7 ±3.2	785.8 ±17.9	338.0 ±7.5	163.2 ±5.8	26.2 ±2.7	1313.2
	MF2	22.4 ±1.1	107.6 ±3.7	104.0 ±4.8	123.2 ±4.5	695.5 ±23.5	345.9 ±8.7	141.0 ±4.1	25.4 ±1.1	1207.8
	MF3	24.8 ±1.4	106.5 ±3.0	107.0 ±3.9	122.5 ±6.0	662.3 ±27.4	308.3 ±6.1	136.8 ±2.8	27.1 ±3.8	1134.5
	MF4	23.1 ±0.9	104.4 ±3.1	106.7 ±2.7	123.2 ±1.7	709.5 ±18.3	341.2 ±12.8	143.4 ±5.2	26.4 ±2.1	1220.5
	MF5	24.6 ±0.7	102.6 ±2.7	104.1 ±5.1	121.1 ±3.5	672.4 ±12.6	278.0 ±4.7	130.5 ±4.3	26.9 ±1.7	1107.8
	L1	24.3 ±0.8	105.4 ±4.0	104.8 ±3.6	111.5 ±4.9	667.7 ±17.5	295.0 ±15.7	136.2 ±3.8	25.1 ±1.2	1124.0
	L2	22.6 ±1.1	105.9 ±3.2	104.9 ±4.2	119.9 ±4.2	684.1 ±8.6	260.9 ±10.1	145.7 ±5.2	28.3 ±1.5	1119.0
	L3	24.1 ±0.6	105.3 ±2.7	105.3 ±1.8	117.2 ±3.7	674.3 ±32.5	234.4 ±8.4	137.9 ±2.7	26.1 ±2.0	1072.7

hba-d: hydroxybenzoic-acid derivatives; flava-d: flavanol derivatives; hca-d: hydroxycinnamic-acid derivatives; flavo-d: flavonol derivatives
MF1-5: main fermentation day 1-5; L1-3: lagering week 1-3

5.6 Study of targeted phenolic compounds, total flavonoid content, total polyphenol content, and DPPH radical scavenging activity during brewing

In this section targeted phenolic compounds were investigated which, according to the literature, are present in the most significant amount in grains and are the most widely studied. With this method we can get a different picture of certain phenolic compounds in raw materials and during the technological steps of brewing. In this section first the effect of adjuncts and special malts, then the effect of infusion and decoction brewing and finally the effect of high gravity brewing is investigated.

5.6.1 The effect of adjuncts and special malts on targeted phenolic compounds, total flavonoid content, total polyphenol content, and DPPH radical scavenging activity of congress worts

This provides an opportunity to examine the effect of using different unmalted cereals and special malts on the selected phenolic compounds during the production of congress worts. For better comparability of worts with different extract contents, all results were calculated relative to 1g of extract. The wort produced from 100% Pilsner malt was considered as a control sample.
Regarding total flavonoid content (Table 21), the use of rice and corn as adjuncts resulted in the largest decrease while wheat, barley, caramel, and roasted malts had a lesser impact on it. Similar to the HPLC results (Table 22), barley and special malts were outstanding. The results of total flavonoid content showed a good correlation with the results of HPLC. A disadvantage of this method is that it is based on the formation of an aluminium-polyphenol complex, but the Maillard reaction products can form a conglomerate with aluminium, giving a false signal in the case of samples containing MRP (e.g., special malts) (ARIMI et al. 2015).
Regarding total polyphenol content (Table 21), the wort produced from 100% roasted malt showed the highest level, followed by the wort of 100% caramel malt and barley grist + roasted malt. In this case, these results may be due to the fact that the TPC method is based on the reduction of Folin-Ciocalteu reagent, which is nonspecific for phenolic compounds because it can be reduced by many non-phenolic compounds such as MRPs and reduction products originating from caramel and roasted malts (HUANG et al. 2005).
In the case of DPPH scavenging activity (Table 21), the wort of 100% roasted malt showed a radical difference. Caramel and roasted malt contributed to the higher results when added to barley or Pilsner malt. This reaction is based on electron transfer, and the MRPs and reduction products that are formed during the roasting process of caramel and roasted malts can participate

in the reaction with DPPH free radicals. (HUANG et al. 2005) The use of wheat and corn as adjuncts decreased the DPPH radical scavenging activity to the greatest extent while rice and barley had a lower impact on it.

Table 21: Total flavonoid content (TFC), Total Polyphenol Content (TPC) and DPPH radical scavenging activity (DPPH) of congress worts.
The results are given in *µg Catechin Equivalent/g extract* of TFC, µg *Gallic Acid Equivalent/g extract* of TPC and *µg Trolox Equivalent/g extract* of DPPH. (mean, sd, n=3)

	TFC	TPC	DPPH
Pils	273.0±21.0	2452.5±7.9	665.2±18.3
Pils+barley	256.0±7.8	1753.3±11.4	626.7±16.1
Pils+rice	54.4±14.1	1839.2±11.6	652.1±52.8
Pils+corn	107.8±15.5	1627.4±3.3	575.3±28.2
Pils+wheat	209.4±18.9	1670.2±8.2	580.2±21.8
Pils+caramel	291.8±29.7	2495.4±18.5	771.9±19.9
Pils+roasted	249.0±22.1	2891.0±17.9	1358.2±55.7
Barley	856.3±49.5	4150.9±114.9	1851.3±99.1
Barley+caramel	770.1±52.5	4277.7±110.9	1618.2±29.6
Barley+roasted	982.0±68.8	8562.3±281.0	2406.3±99.4
Caramel	636.1±51.6	8438.4±58.5	3138.7±82.6
Roasted	790.3±63.2	13675.9±371.8	11009.1±404.0

Regarding phenolic acid content (Table 22) 100% barley wort showed the highest results and also contained high concentrations of catechin, epicatechin and procyanidin B3. This result had various causes: firstly, unlike the other studied adjuncts, barley has a husk which, according to QUINDE-AXTELL et al. (2006), contains high levels of phenolic compounds; secondly, in this section the free forms of phenolic compounds were investigated, on which the malting process, primarily kilning, can have a negative effect resulting in a lower concentration in malted barley compared to the unmalted (DVOŘÁKOVÁ et al. 2008b); thirdly, it is important to stress that these results are related to extract content and, as can be seen in Table 3 (page 51) the wort made from barley had the lowest extract content. This was because congress mashing as a hot water extraction did not result in a high extract content due to the lack of endosperm modification, which occurs during malting, and the lack of enzymes, but was effective in the release of free phenolic substances mainly from the outer layers of the kernel. It is interesting that barley, despite its high phenolic acid content, when used as an adjunct added to Pilsner malt, only slightly increased the p-hydroxybenzoic acid and vanillic acid concentrations and decreased the trans-ferulic acid concentration compared to the 100% Pilsner wort. This suggests that the enzymatic activity of Pilsner malt did not contribute to a more efficient dissolution of free phenolic compounds in the wort when used at 50-50% w/w with barley. Using 50% Pilsner malt added to barley grist resulted in a higher extract content compared to 100% barley wort due to

the enzymatic activity of the malt, on the other hand the concentration of phenolic acids is lower in 50-50% w/w Pilsner malt-barley wort compared to 100% barley wort. Regarding the worts made from 100% caramel and 100% roasted malts, they did not contain free p-hydroxybenzoic acid or vanillic acid and had the lowest concentration of trans-ferulic acid.

Table 22: Free phenolic composition of the congress worts (mean, sd, n=2).
The results are given in *µg/g extract*.

	4-hba	va	c	ec	pcB3	tfa
Pils	0.39±0.02	2.30±0.58	4.49±.047	8.01±1.87	3.62±0.74	14.20±1.86
Pils+barley	0.45±0.01	2.46±0.03	9.52±1.46	3.87±1.00	3.81±0.15	7.79±1.45
Pils+rice	0.50±0.05	1.63±0.36	1.31±0.05	8.76±1.10	1.58±0.43	9.02±1.83
Pils+corn	0.24±0.01	1.82±0.08	5.31±0.34	8.79±1.26	1.69±0.33	9.86±1.44
Pils+wheat	0.26±0.02	2.26±0.63	1.09±0.07	3.73±.041	2.01±.0.4	7.37±0.69
Pils+caramel	0.44±0.01	2.25±0.58	9.36±1.64	11.31±1.41	5.94±0.48	11.86±0.66
Pils+roasted	0.34±0.01	2.05±0.40	7.61±1.03	9.59±1.84	5.96±0.79	10.52±0.07
Barley	3.96±0.10	13.93±0.01	27.74±1.84	13.32±0.74	12.26±1.61	15.98±0.15
Barley+caramel	3.66±0.28	9.83±0.85	40.11±3.34	7.84±0.44	7.80±1.40	14.30±2.96
Barley+roasted	3.73±0.26	10.77±1.35	39.33±2.22	10.54±1.33	17.18±3.33	12.65±0.60
Caramel	n.d.	n.d.	52.80±5.71	81.51±7.45	n.d.	4.23±0.16
Roasted	n.d.	n.d.	27.05±3.05	n.d.	n.d.	5.07±.038

4-hba: 4-hydroxybenzoic acid; va: vanillic acid; c: (+)-catechin; ec: (-)-epicatechin; pcB3: procyanidin B3; tfa: trans-ferulic acid; n.d.: not detected.

According to TIAN et al. (2004), and HERNÁNDEZ et al. (2011), the major phenolic compounds of wheat and rice are phenolic acids, while for corn, flavonoids, and phenolic acids (ŽILIĆ et al. 2012). The use of these adjuncts did lead to changes in the composition of phenolics compared to the wort produced from Pilsner malt.

The content of p-hydroxybenzoic acid increased with rice as an adjunct, while with corn and wheat, it decreased. Barley and special malts did not have any significant influence on this compound.

The levels of vanillic acid with rice and corn adjuncts were the lowest. Wheat and barley did not have any influence on the concentration of vanillic acid. The use of adjuncts and special malts resulted in a decrease in trans-ferulic acid content compared to the Pilsner wort.

The wort produced from 100% caramel malt had the highest catechin and epicatechin content. The wort produced from 100% roasted malt also contained a relatively high concentration of catechin but epicatechin was not detected. Neither 100% caramel nor 100% roasted worts contained procyanidin B3, but, when added to Pilsner malt, an increase in the concentration of procyanidin B3 was observed, while when added to barley, caramel malt decreased, and roasted malt increased the procyanidin B3 concentration. The special malts contributed to the increase in catechin and epicatechin levels when added to Pilsner malt or barley. CHANDRASEKARA et

al. (2011) reported a 2-4-fold higher flavonoid content in cashew nuts treated at high temperature compared to the raw nut. These results suggest liberation and isomerization of such compounds during heat treatment. This further lends support to the observed decrease in tannin content. Furthermore, YU et al. (2006) showed that roasting decreased the proanthocyanidin (trimers and tetramers) content of peanut skins and increased its monomer content when compared to the raw skin. CALIGIANI et al. (2007) found that roasting of Ghana cocoa bean at 150–220°C for 20 minutes decreased epicatechin by 78.5% and increased catechin by 63%.

The use of wheat decreased the catechin and epicatechin content of the wort to the greatest extent, due to its low flavonoid content (HERNÁNDEZ et al. 2011). Rice decreased catechin but increased the epicatechin concentration compared to Pilsner wort. Corn, due to its high flavonoid content (ŽILIĆ et al. 2012), increased both the catechin and epicatechin concentrations compared to Pilsner wort.

Among unmalted grains barley wort had the highest concentration of procyanidin B3, and all other adjuncts added to Pilsner malt caused a decrease. The lower concentration of procyanidin B3 in Pilsner wort compared to the wort made from barley agrees with the results of ZHAO et al. (2006) who reported a decrease due to malting.

5.6.2 Comparison of the effect of infusion and decoction mashing on targeted phenolic compounds, total flavonoid content, total phenolic content, and DPPH radical scavenging activity

In brewing practice there are two main mashing techniques used in the brewhouse: infusion mashing and decoction mashing. To compare the effect of the different techniques on antioxidant activity and phenolic compounds worts were produced with 3 different mashing techniques (page 52) at a pilot scale brewing equipment. The main difference between the two decoction mashings is the proportion of mash separated for boiling, it was one third during Decoction 1 and two third during Decoction 2. Samples were not taken during mashing only after wort separation from the first worts, second wort (sparging), and sweet wort.

Regarding total flavonoid content, samples of Decoction 2 have the highest values in case of both the first wort, sparging and sweet wort (Table 23).

DPPH radical scavenging activity shows similar results (Table 23). An increasing tendency can be observed during the whole process in case of mashing Decoction 1. Decoction 2 and Infusion have higher results of the sparging than of the sweet worts. Samples of Decoction 2 have the highest results at each stage while Decoction 1 has the lowest values.

With respect to TPC there are small differences between both the samples produced with different mashing technologies and between the results of different stages of the technology (Table 23). Samples of mashing Decoction 2 have the highest and Infusion the lowest values through the whole process. These results are due to the formation of MRPs and reductones due to the thermal impact, as Decoction 2 underwent the highest degree of heat treatment while Infusion underwent the lowest degree of heat treatment resulting in the highest and lowest antioxidant activity among these samples.

It is interesting that sparging has the highest values in most cases for all the three methods, highlighting the importance of this technological step for antioxidant activity.

With respect to the results of the sweet worts it seems that decoction technology contributes to higher antioxidant activity in the wort.

Table 23: Total flavonoid content (TFC), Total Polyphenol Content (TPC) and DPPH radical scavenging activity (DPPH) of worts produced with different mashing technology.
The results are given in *µg Catechin Equivalent/g extract* of TFC, *µg Gallic Acid Equivalent/g extract* of TPC and *µg Trolox Equivalent/g extract* of DPPH. (mean, sd, n=3).

		TFC	TPC	DPPH
Infusion	**FW**	278.3±10.4	3327.3±112.4	1323.5±34.3
	S	319.8±13.2	3556.4±134.5	1604.0±17.1
	SW	296.6±8.6	3380.5±65.4	1489.4±20.1
Decoction 1	**FW**	299.7±17.1	3378.9±37.2	1189.0±22.0
	S	351.2±6.2	3675.0±142.5	1419.1±13.5
	SW	289.0±5.4	3519.2±74.5	1548.6±24.8
Decoction 2	**FW**	389.7±6.7	3389.2±63.2	1562.1±10.0
	S	416.2±7.9	3661.5±74.5	1811.8±11.7
	SW	421.1±3.7	3557.1±32.5	1674.4±12.8

FW: first wort; S: sparging; SW: sweet wort

The result of the phenolic composition of worts are shown in Table 24.

Regarding vanillic acid content there are minor differences between the samples of the three different mashing techniques at certain technological steps, suggesting similar release efficiency of the different mashing technologies.

With respect to trans-ferulic acid, Infusion has the highest concentration among first wort samples, while Decoction 1 and 2 have lower trans-ferulic acid content. Among sparging samples Decoction 2 has the lowest value, while Infusion and Decoction 1 have higher values.

Between sweet worts there are smaller differences, the range is 7.45- 8.83 µg/g extract. The infusion technology seems to be the most effective in recovering trans-ferulic acid. It is probably due to the lower heat impact compared to Decoction mashings where a boiling step is applied and can induce the loss of trans-ferulic acid content.

Regarding (+)-catechin content, samples of Decoction 2 have the lowest values during the whole process. There are big differences between the different mashing technologies. In case of first worts Decoction 1 has the highest concentration, regarding spargings Infusion and Decoction 1 have the highest values with almost the same concentrations. It is important to stress that in case of Infusion and Decoction 1 mashing sparging could dissolve even higher concentration of (+)-catechin than the first wort. In case of sweet worts Infusion has the highest concentration. According to CHANDARSEKARA (2011) liberation of flavonoids due to high temperature treatment can cause 2-4-fold increase of flavonoid monomer compounds while according to YU (2006) the concentration of proanthocyanidin trimers and tetramers decrease, based on these observations, higher concentration of (+)-catechin and (-)-epicatechin would have been expected for decoction mashings than infusion.

With respect to (-)-epicatechin content, samples of Infusion have the highest values during the whole process while Decoction mashings have lower and similar results at each technological point. The biggest differences are observed in case of the sparging samples. The Infusion mashing technology seems to be the most effective in recovering the (-)-epicatechin content.

Table 24: Free phenolic composition of worts produced with different mashing technology (mean, sd, n=2). The results are given in *µg/g extract*.

		4-hba	va	c	ec	pcB3	tfa
Infusion	**FW**	n.d.	0.70±0.02	24.56±1.16	19.63±1.02	21.24±0.72	10.60±0.27
	S	n.d.	0.74±0.03	36.81±0.82	22.79±0.97	23.51±1.23	10.24±0.26
	SW	n.d.	0.53±0.10	27.43±2.44	20.25±1.16	13.15±1.15	8.83±0.44
Decoction 1	**FW**	n.d.	0.67±0.07	28.56±1.02	18.51±1.38	13.08±2.32	8.64±1.02
	S	n.d.	0.63±0.06	36.79±0.88	17.23±0.71	12.74±1.87	10.03±0.83
	SW	n.d.	0.55±0.07	26.11±2.11	18.67±2.15	10.48±1.61	7.45±0.44
Decoction 2	**FW**	n.d.	0.68±0.08	21.20±2.22	19.04±1.33	22.80±1.56	8.23±0.62
	S	n.d.	0.69±0.11	20.24±2.06	17.23±1.15	18.97±1.84	8.06±0.50
	SW	n.d.	0.54±0.05	21.77±1.12	17.77±1.11	15.68±0.82	8.19±0.50

4-hba: 4-hydroxybenzoic acid; va: vanillic acid; c: (+)-catechin; ec: (-)-epicatechin; pcB3: procyanidin B3; tfa: trans-ferulic acid; n.d.: not detected.
FW: first wort; S: sparging; SW: sweet wort

Regarding procyanidin B3 there are big differences between both the different mashing technologies and the samples taken at different steps of the mashing processes. Aware of the haze active property of procyanidin B3 it would have been expected to form protein-polyphenol complexes more extensively in the samples produced with decoction technology compared to infusion technology resulting in lower concentrations of this compound (ARON et al. 2010), but this tendency cannot be observed. The samples of Decoction 1 have the lowest values during the whole process. The procyanidin B3 concentration shows a decreasing tendency during the whole process of decoction mashings. Infusion shows similar tendency except for the sparging which has slightly higher concentration than the first wort. The lower values of sweet worts compared to the first or second worts are due to the timing of sampling as the samples were taken after the boiling had already started to homogenize the wort and part of the procyanidin B3 content may already been precipitated with proteins.

5.6.3 The effect of high gravity brewing on targeted phenolic compounds, total flavonoid content and total phenolic content

Today, due to consideration of economy, most large breweries make some of their products using so-called high gravity brewing technology. This means that in the brewhouse a more concentrated wort is brewed resulting a final beer with higher alcohol content. After fermentation this concentrated beer is diluted (and carbonated) usually during filtration.
Regarding TFC and TPC results of the diluted and the not diluted 9 %m/m original extract beers there is no significant difference between the two samples. On the other hand, it is shown on Table 25 that there are significant differences between the phenolic content of the two samples. The beer which is originally brewed to 9 %m/m original extract and had not been diluted contains significantly higher amount of all investigated phenolic compounds than the beer which was brewed to 14 %m/m and then diluted to 9 %m/m original extract. This suggests that during the whole process the degree of dissolution of phenolic compounds depends on the extract content, for beers with higher extract content, the dissolution of phenolic compounds is proportionally lower. This means that beers which are brewed applying high gravity brewing and thus a high degree of dilution, contains lower amount of phenolic compounds that beers brewed by regular technology. From a technological point of view, it has advantages due to the negative effect of phenols on colloidal stability and aging of the beer, on the other hand from a nutritional point of view this may have the disadvantage of containing fewer compounds that have a positive effect on human health.

Table 25: Free phenolic composition (mean, sd, n=2), Total flavonoid content (TFC), Total Polyphenol Content (TPC) and DPPH radical scavenging activity (DPPH) (mean, sd, n=3) of high gravity and normal beer.

The results are given in *µg/g extract of phenolic compounds*, *µg Catechin Equivalent/g extract* of TFC, *µg Gallic Acid Equivalent/g extract* of TPC and *µg Trolox Equivalent/g extract* of DPPH.

	4-hba*	va*	c*	ec*	pcB3*	tfa*	TFC	TPC
High gravity beer	0.19±0.02	0.25±0.03	3.11±0.12	1.22±0.03	0.95±0.03	0.87±0.04	57.7±1.7	173.9±4.1
Normal beer	0.29±0.01	0.39±0.04	4.53±0.21	1.67±0.12	1.01±0.08	1.17±0.09	54.4±2.3	172.3±3.5

4-hba: 4-hydroxybenzoic acid; va: vanillic acid; c: (+)-catechin; ec: (-)-epicatechin; pcB3: procyanidin B3; tfa: trans-ferulic acid; *the difference is significant ($p<0.05$)

6. CONCLUSIONS AND RECOMMENDATIONS

Study of folate content during malting

Based on my results, it can be stated that the folate content of pale barley malt is outstanding among the malts made from different grains. There is considerable difference between the folate content of spring and winter barleys of different vintages, but in one vintage the spring and in the other the winter cultivar had higher folate content. The folate content of malts produced from winter barley was higher both under micro and industrial scale. Malting increases the folate content of malts of different types and vintages of barleys, which is mainly due to the activity of the embryo and the enzymatic degradation processes during malting. The biggest difference between micro and industrial scale malting was the trend during the malting process, even though the same parameters were used during the processes. Barleys malted at micro scale reached their highest value on the 3^{rd}-5^{th} days of malting, and then their folate content decreased due to kilning. In contrast, barleys malted at industrial scale showed a steadily increasing trend, reaching the highest folate content in the finished malt. Based on the evolution of folate content malting can be divided into two stages. There is a formation phase that is well observed at both micro and industrial scales and is caused by the fact that folate is produced by the embryo because it is required for growth and cell differentiation. It is an internal, gene-dictated process, it does not affect the tendency of production scale. It is affected by its extent, because the controllability of parameters is different in micro and industrial scales, and the production volume also affects how much heat or moisture each grain receives. In the second stage, the change in the amount of folate is determined only by an external effect, namely heat treatment. Here only this external influence affects, not only the extent but also the trend. The roasting experiment proved that the folate in barley malt is still stable at temperatures around 100 °C, but above that folate begins to degrade and is in inverse relationship with temperature and time. At 200 °C folate content drops to one third in just 5 minutes, proving that special malts that are exposed to higher temperatures are not good sources of folate.

Evaluating the results from the point of view of nutrition, it can be stated that the folate content of barley can be increased up to 10 times by germination, which can be preserved by gentle kilning. When kilning, it is important that the temperature does not exceed 100 °C. In terms of folate content, pale barley malt is the best choice for further processing in the food industry.

Study of folate content during brewhouse processes

During mashing, prolonged protein rest had no effect on folate content. The folate content of the mash increased continuously, which is due to the enzymatic processes. By the end of the first enzyme rest (protease rest), a significant part of the folate is already dissolved. During wort separation, sparging is of paramount importance, which, related to extract content, contained at least the concentration of folate as the first wort. Hop boiling, at which a decrease would have been expected in folate content, did not cause a decrease.

The brewing process is effective in recovering the folate content of malt, so brewed drinks have the potential to contribute considerably to daily folic acid intake. Based on my dissertation, it can be stated that malt beverages or non-alcoholic beers can be produced with a significant folate content if choosing the appropriate raw materials. On the other hand, the metabolism of the yeast greatly influences the development of the folate content, so further studies would be needed on the production of beers with normal alcohol content, which investigate specifically the processes that take place during fermentation, in order to produce beers with higher folate content.

Antioxidant activity and total phenolic composition from grains to malt

From the point of view of antioxidant activity and phenolic content, barley malt is also of outstanding importance similarly as in case of folate content, however, it is not the pale barley malt, but the special caramel and coloring malt, primarily due to their very high flavanol derivative content.

There is no real correlation between the antioxidant activity and the phenolic content of barleys, as those with lower antioxidant activity have a higher phenolic content and vice versa. In terms of phenolic content, according to cultivar, spring barleys have a higher concentration, and according to vintage, 2018 barleys have a higher concentration.

The trend during malting is similar to the folate content of micro scale malts in terms of both antioxidant activity and phenolic content. The antioxidant activity and phenolic content of both micro and industrial scale malts reach a peak during germination and then decreases with kilning.

As a result of roasting, the antioxidant activity increased in all cases except for the ABTS method, as did the total phenolic content, which well reflects the results of the preliminary experiment with special malts. The hydroxycinnamic-acid derivative content began to increase intensively around 150 °C and the flavanol derivative content around 175 °C. The flavanol derivative content increased continuously until the longest roasting at the highest temperature, while the other phenolic groups stagnated after the initial growth.

In terms of antioxidant activity and phenolic content, malts roasted at a high temperature are outstanding, which is due to the formation of reductones with antioxidant activity and the structural transformations in the case of phenolic compounds, but this can be negative from the technological point of view, as dark malts contain high concentration of flavanol derivatives which have the ability to form polymers, thereby adversely affecting the colloidal stability of the final product in the case of filtered beers, but may be particularly advantageous for the production of turbid beers. In addition, the antioxidant activity of malts treated at higher temperature is higher, which can improve the oxidative stability of beers.

Antioxidant activity and total phenolic composition of hops

Great differences were found between the different types of hops in terms of both antioxidant activity and phenolic content. No correlations were found between antioxidant activity, phenolic content, and alpha-acid content. It can be stated that the antioxidant activity and phenolic content of hops is high, however, there are significant differences in phenolic composition. Hops can be chosen for brewing keeping in mind the quality requirements of the final product to be produced. If unfiltered, more turbid product is brewed, the right choice is the hop with high flavanol derivative content, if a filtered product is brewed, the right choice is the hop with lower flavanol derivative content.

Study of antioxidant activity and total phenolic composition during brewing

During mashing, different degrees of change occur in pale and dark laboratory and pilot scale samples, however, during mashing, the antioxidant activity and polyphenol content increase in all cases. The first wort separation has a small negative effect on both antioxidant activity and phenolic content in all cases. It should be emphasized that sparging showed high values in terms of both antioxidant activity and phenolic content related to extract content.

Hop boiling caused a decrease in phenolic content in pale worts, but an increase in dark worts. This may be due to the protective effect of reductones originating from special malts.

During fermentation, the flavanol derivative content shows the greatest decrease, which can be positive for the colloidal stability of the final product.

The higher antioxidant activity and phenolic content of the dark malts used is also reflected in the brewhouse process. The antioxidant activity and phenolic composition of the wort and beer can be influenced with the selection of the right amount and type of special malts, considering the desired quality of the final product.

Effect of adjuncts on antioxidant activity and targeted phenolic content

Rice, corn, and wheat used as adjuncts decrease the free phenolic acid content of worts. This is a negative finding from both the nutritional point of view and technology because these compounds can contribute to the longer shelf life of a product and the maintenance of antioxidant/free radical balance in our body. On the other hand, when using these adjuncts, the concentration of procyanidin B3 decreased, which is beneficial for the better colloidal stability of the final product. Barley can contribute to a higher phenolic acid content but must be treated carefully from the technological point of view because its flavanol content (catechin, epicatechin, procyanidin B3) is also high. On the other hand, there is an advantage in the use of barley compared to other adjuncts; the presence of husk allows easier wort separation.

Special malts have a high flavanol content in line with the previous results, which clearly shows that this high flavanol content is mainly due to the monomers (catechin, epicatechin) and not to the dimers (procyanidin B3).

Effect of infusion and decoction mashing on antioxidant activity and targeted phenolic content

Sparging has the highest values of antioxidant activity in most cases among first wort, sparging and sweet wort samples, highlighting the importance of this technological step for antioxidant activity. Decoction mashing technology contributes to higher antioxidant activity of worts, but the same cannot be stated for the phenolic content.

Effect of high gravity brewing on antioxidant activity and targeted phenolic content

No significant difference was observed between the antioxidant activity of high gravity brewed and normal beer, on the other hand all the investigated phenolic compounds were significantly higher in the normal beer which was not diluted. High gravity brewing decreases the phenolic content in beer, it can result in worse flavor stability and a less full taste, but the colloid stability of the product may be better.

It can be stated that the study of both folate content, antioxidant activity and phenolic content during malting and brewing is extremely complex. The type, the sowing and harvesting time of the raw materials, the way of their processing, and proportion of use, the variability of many parameters of the brewing process and the diversity of the final products offers endless research opportunities, not to mention the diversity of the analytes.

7. NEW SCIENTIFIC RESULTS

1. I have demonstrated that significant part of the folate content of malt is released during the 20 minutes at 52 °C protease rest of the mashing process of brewery wort production. I have proved that a prolonged protease rest (40 minutes at 52°C) does not increase the folate content of mash. Furthermore, I have demonstrated that folate content of the mash increases continuously until the end of the mashing process (20 minutes at 52°C, 45 minutes at 62°C and 15 minutes at 72°C).

2. I have proved that the hop boiling process (one hour at 100 °C) does not result in the decrease of the folate content of wort.

3. I have proved that caramel and coloring malts have outstanding flavanol derivative content. The high flavanol content is mainly due to monomers (catechin and epicatechin) rather than dimers (procyanidin B3).

4. I have demonstrated that antioxidant activity and phenolic content reach a peak during the technological step of germination of the malting process of barley.

5. I have demonstrated that roasting at 100, 125, 150 or 175 °C for 20 minutes and at 200 °C for 10-15 minutes increases the antioxidant activity and total phenolic content of malt.

6. I have demonstrated that the antioxidant activity and phenolic content of mash increase during the mashing process (20 minutes at 52°C, 45 minutes at 62°C and 15 minutes at 72°C). I have proved that decoction mashing technology result in wort with higher antioxidant activity than infusion mashing technology.

7. I proved that the technological step of sparging has an outstanding contribution to the antioxidant activity, phenolic content, and folate content of wort.

8. I have proved that beer produced with high gravity brewing technology contains less phenolic compounds as opposed to beer produced with normal gravity brewing technology.

9. I have proved that flavanol derivative content decreases significantly during the fermentation process (primary fermentation at 16°C for 5 days and secondary fermentation at 3°C for 21 days with ale type brewing yeast).

8. SUMMARY

In recent years, the lifestyle of people, especially in western countries, has changed, we are pursuing an accelerated way of life, which also affects our eating habits. Processed foods make up an increasing proportion of the food consumed. As a result of processing, they lose their nutritional value, primarily their biologically active nutrients. Intake of biologically active nutrients such as vitamins and antioxidants are essential for the proper functioning of the body. To know how we can meet our body's need for vitamins and antioxidants, we also need to know how the processing of raw materials during food production affects these components. My dissertation is intended to help answer this question regarding malting and brewing.

Malting and brewing are centuries old technologies that may seem simple, yet very complex processes. The raw materials and parameters used in each technological step affect the later stages of the process and the chemical properties and quality of the final product.

Evolution of folate during malting and brewing

Cereals are considered a good source of folate. The folate content of beer originates from two main sources: grains and yeast. As more than 90% of commercial beers are filtered, grains are the primary source of folate in the majority of the final products. The most often used cereal for beer brewing is barley but wheat, oat and rye are also applied besides barley. Grains used for brewing are mainly used in their malted form.

To study the effect of **malting** on folate content, first malts produced from different grains were investigated, then malting was carried out on micro and industrial scale which was followed by a roasting experiment.

Based on my results, it can be stated that the folate content of pale barley malt is outstanding among the malts made from different grains. There is considerable difference between the folate content of spring and winter barleys of different vintages. The folate content of malts produced from winter barley was higher both under micro and industrial scale. Malting increased the folate content of malts of different types and vintages of barleys, which is mainly due to the activity of the embryo and the enzymatic degradation processes during malting. The biggest difference between micro and industrial scale malting was the trend during the malting process, even though the same parameters were used during the processes. Barleys malted at micro scale reached their highest value on the 3^{rd}-5^{th} days of malting, and then their folate content decreased due to kilning. In contrast, barleys malted at industrial scale showed a steadily increasing trend, reaching the highest folate content in the finished malt.

The **roasting** experiment proved that the folate in barley malt was still stable at temperatures around 100 °C, but above that folate began to degrade and is in inverse relationship with temperature and time. At 200 °C folate content dropped to one third in just 5 minutes, proving that special malts that are exposed to higher temperatures are not good sources of folate.
Evaluating the results from the point of view of nutrition, it can be stated that the folate content of barley can be increased up to 10 times by germination, which can be preserved by gentle kilning. When kilning, it is important that the temperature does not exceed 100 °C. In terms of folate content, pale barley malt is the best choice for further processing in the food industry.

To study the effect of **brewing** on folate content worts were produced on laboratory and pilot scale.
During mashing, prolonged protein rest had no effect on folate content. The folate content of the mash increased continuously, which is due to the enzymatic processes. By the end of the first enzyme rest (protease rest), a significant part of the folate was already dissolved. During wort separation, sparging is of paramount importance, which, related to extract content, contained at least the concentration of folate as the first wort. Hop boiling, at which a decrease would have been expected in folate content, did not cause a decrease.
The brewing process is effective in recovering the folate content of malt, so brewed drinks have the potential to contribute considerably to daily folic acid intake. Based on my results, it can be stated that malt beverages or non-alcoholic beers can be produced with a significant folate content if choosing the appropriate raw materials. On the other hand, the metabolism of the yeast greatly influences the development of the folate content, so further studies would be needed on the production of beers with normal alcohol content, which investigate specifically the processes that take place during fermentation, in order to produce beers with higher folate content.

Evolution of antioxidant activity and phenolic composition during malting and brewing

In addition to folates, antioxidant compounds are the other group of health-promoting components studied in the dissertation. It has been a hot topic in the last twenty years and has been studied widely even during certain steps of brewing and malting but a comprehensive study, which includes raw materials and technologies that have not been investigated so far, can make the existing knowledge even more valuable. Polyphenols and antioxidants influence, directly or indirectly, beer production because of their chemical properties and, in particular, because of their multiplicative reactivity. Polyphenols can form many different compounds.

Monomer and oligomer polyphenols can interact both in the aerobic and in anaerobic phase during the brewing process.

Antioxidant activity and phenolic composition during malting and roasting

Like in case of folate experiments, first the antioxidant activity and phenolic composition of malts produced from different grains were investigated, then the evolution of these compounds was studied during micro and industrial scale **malting** followed by roasting experiments.

From the point of view of antioxidant activity and phenolic content, barley malt is also of outstanding importance among different cereals, however, it is not the pale barley malt, but the special caramel and coloring malt, primarily due to their very high flavanol derivative content.

There was no real correlation found between the antioxidant activity and the phenolic content of barleys. In terms of phenolic content, according to cultivar, spring barleys had a higher concentration, and according to vintage, 2018 barleys had a higher concentration compared to the 2017 crop.

The antioxidant activity and phenolic content of both micro and industrial scale malts reached a peak during germination and then decreased with kilning. As a result of roasting, the antioxidant activity increased in all cases, as did the total phenolic content, which well reflects the results of the preliminary experiment with special malts. The hydroxycinnamic-acid derivative content began to increase intensively around 150 °C and the flavanol derivative content around 175 °C. The flavanol derivative content increased continuously until the longest roasting at the highest temperature, while the other phenolic groups stagnated after the initial growth.

Malts **roasted** at a high temperature are outstanding, which is due to the formation of reductones with antioxidant activity and the structural transformations in the case of phenolic compounds, but this can be negative from the technological point of view, as dark malts contain high concentration of flavanol derivatives which have the ability to form polymers, thereby adversely affecting the colloidal stability of the final product but may be particularly advantageous for the production of turbid beers. In addition, the antioxidant activity of malts treated at higher temperature is higher, which can improve the oxidative stability of beers.

Antioxidant activity and phenolic composition of hops

Hop is the other important raw material for brewing in terms of antioxidant activity and phenolic content. It contains higher concentrations of polyphenols than barley malt (barley malt 50–100 mg/100 g, hops up to 4 g/100 g), but contributes to only 20-30% of total polyphenols in traditional beers. Hop cones contain 3% to 6% polyphenolic compounds related to dry weight.

In the study 16 different types of hops with different alpha-acid content were involved.
Great differences were found between the different types of hops in terms of both antioxidant activity and phenolic content. No correlations were found between antioxidant activity, phenolic content, and alpha-acid content. It can be stated that the antioxidant activity and phenolic content of hops are high, however, there are significant differences in phenolic composition. Hops can be chosen for brewing keeping in mind the quality requirements of the final product to be produced. If an unfiltered, turbid product is brewed, the right choice is the hop with high flavanol derivative content, if a filtered product is brewed, the right choice is the hop with lower flavanol derivative content.

Antioxidant activity and phenolic composition during the brewing process

For **brewing** experiments a pale and a dark wort were brewed on laboratory scale, furthermore on pilot scale a pale and a dark beer were produced.
During mashing, different degrees of change occurred in pale and dark laboratory and pilot scale samples, however, the antioxidant activity and polyphenol content increased in all cases. The first wort separation had a small negative effect on both antioxidant activity and phenolic content in all cases. It should be emphasized that sparging showed high values in terms of both antioxidant activity and phenolic content related to extract content. Hop boiling caused a decrease in phenolic content in pale worts, but an increase in dark worts. This may be due to the protective effect of reductones originating from special malts. During fermentation, the flavanol derivative content showed the greatest decrease, which can be positive for the colloidal stability of the final product in case of a filtered beer.
The higher antioxidant activity and phenolic content of the dark malts used are also reflected in the brewhouse process. The antioxidant activity and phenolic composition of the wort and beer can be influenced with the selection of the right amount and type of special malts, considering the desired quality of the final product.

Effect of adjuncts on the antioxidant activity and targeted phenolic compounds of congress worts

Due to the competitiveness of the beer market, breweries are constantly under pressure to produce consistently high-quality beer at a lower cost. To achieve this goal, brewers have increasingly replaced malt with various less expensive adjuncts. It is estimated that up to 85-90% of beer worldwide is now produced with adjuncts. The most commonly used adjunct materials are corn (46% of total adjunct), rice (31%), barley (1%), wheat (>1%) and sugars and syrups (22%).

In order to study the effect of adjuncts on antioxidant activity and phenolic composition of worts, congress worts were produced on a laboratory mashing equipment.

Rice, corn, and wheat used as adjuncts decreased the free phenolic acid content of worts. This is a negative finding from both the nutritional point of view and technology because these compounds can contribute to the longer shelf life of a product and the maintenance of antioxidant/free radical balance in our body. On the other hand, when using these adjuncts, the concentration of procyanidin B3 decreased, which is beneficial for the better colloidal stability of the final product. Barley contributed to a higher phenolic acid content but must be treated carefully from the technological point of view because its flavanol content (catechin, epicatechin, procyanidin B3) was also high. Special malts had a high flavanol content which is in line with the previous results, which clearly showed that this high flavanol content is mainly due to the monomers (catechin, epicatechin) and not to the dimers (procyanidin B3).

Study of the effect of decoction and infusion mashing technology on antioxidant activity and targeted phenolic compounds

Over the course of time many different mashing processes have been developed, which can in general be divided into infusion and decoction methods. During infusion technology the temperature of the whole amount of mash is constantly raised, keeping enzymatic rests at certain temperatures. The other is decoction technology where a part of the mash is separated and boiled, thereafter added back to the other part. The choice of infusion (enzymatic digestion) or decoction (enzymatic and physical digestion) is an important mash parameter.

In order to study the effect of infusion and decoction mashing on antioxidant activity and phenolic content one infusion mash and two decoction mashes were produced on pilot scale.

Among first wort, sparging and sweet wort samples sparging had the highest values of antioxidant activity in most cases, highlighting the importance of this technological step for antioxidant activity and phenolic content. Decoction mashing technology contributed to higher antioxidant activity of worts, but the same cannot be stated for the phenolic content.

Effect of high gravity brewing on antioxidant activity and targeted phenolic compounds

Today, in terms of economy, most large breweries make some of their products applying so-called high gravity brewing technology. This means that in the brewhouse a more concentrated wort is brewed than the extract content of the final beer will be and then after fermentation this concentrated beer is diluted usually during filtration.

In order to study the effect of high gravity brewing two beers were produced from the same raw materials and with the same technology on pilot scale, one was brewed to 9 %m/m original extract content and the other to 14 %m/m original extract content. Before analysis, the 14% beer was diluted to 9% with deoxygenated water.

No significant difference was observed between the antioxidant activity of high gravity brewed and normal beer, on the other hand all the investigated phenolic compounds were significantly higher in the normal beer which was not diluted. High gravity brewing decreased the phenolic content in beer, it can result in worse flavor stability and a less full taste, but the colloid stability of the product may be better.

9. ÖSSZEFOGLALÓ

Az elmúlt években, főleg a nyugati országokban, megváltozott az emberek életmódja, felgyorsult életmódot folytatunk, ami kihat az étkezési szokásainkra is. A feldolgozott élelmiszerek az elfogyasztott ételek egyre nagyobb hányadát teszik ki. Az élelmiszerek a feldolgozás során veszítenek tápértékükből, elsősorban biológiailag aktív tápanyagaikból. A biológiailag aktív tápanyagok, például a vitaminok és az antioxidánsok bevitele elengedhetetlen a szervezet megfelelő működéséhez. Ahhoz, hogy tudjuk, hogyan elégíthetjük ki szervezetünk vitamin- és antioxidánsszükségletét, tudnunk kell azt is, hogy az élelmiszer-előállítás során a nyersanyagok feldolgozása hogyan befolyásolja ezeket az összetevőket. Dolgozatom célja, hogy segítsen megválaszolni ezt a kérdést a malátázás és a sörgyártás terén.

A maláta- és a sörgyártás évszázados technológiák, amelyek egyszerűnek tűnhetnek, ugyanakkor nagyon összetett folyamatok. A felhasznált alapanyagok, az egyes technológiai lépések és az alkalmazott beállítások kihatással vannak az egész folyamatra, a késztermék fizikai-kémiai tulajdonságaira és minőségére is.

A folát tartalom vizsgálata malátázás és sörfőzés során

A gabonaféléket jó folátforrásnak tekintik. A sör foláttartalma két fő forrásból származik: a gabonákból és az élesztőből. Mivel a kereskedelmi sörök több mint 90% -a szűrt, a végtermékek többségében a gabonák az elsődleges folátforrások. A sörfőzéshez leggyakrabban az árpát használják, de az árpa mellett búzát, zabot és rozst is alkalmaznak. A sörfőzéshez elsősorban malátázott formában használják a gabonákat.

A **malátázás** foláttartalomra gyakorolt hatásának vizsgálatához először különböző gabonákból előállított malátákat vizsgáltam, majd félüzemi és nagyüzemi léptékű malátázás hatásait vizsgáltam, amelyet pörkölési kísérlet követett.

Eredményeim alapján megállapítható, hogy a világos árpamaláta foláttartalma kiemelkedő a különböző gabonából készült maláták között. Jelentős különbség van a különböző évjáratú tavaszi és őszi árpák foláttartalma között. Az őszi árpából előállított maláták foláttartalma mind félüzemi, mind nagyüzemi léptékben magasabb volt. A malátázás növelte a különböző típusú és évjáratú árpák folsavtartalmát, ami elsősorban az embrió aktivitásának és a malátázás során bekövetkező enzimatikus lebontási folyamatoknak köszönhető. A legnagyobb különbség a félüzemi és a nagyüzemi léptékű malátázás között a malátázási folyamat során tapasztalható tendencia volt, annak ellenére, hogy ugyanazokat a paramétereket alkalmaztuk a folyamatok során. A félüzemi léptékben malátázott árpák a legmagasabb értéket a csíráztatás 3-5. napján

érték el, majd az aszalás hatására csökkent a foláttartalmuk. Ezzel szemben a nagyüzemi méretben malátázott árpák folyamatosan növekvő tendenciát mutattak, és a kész malátában érték el a legmagasabb foláttartalmat.

A **pörkölési kísérlet** bebizonyította, hogy az árpamalátában lévő folát 100 °C körüli hőmérsékleten még stabil volt, de ezen felül a folát bomlani kezdett. A foláttartalom fordított arányosságban viszonyult a hőmérséklethez és az időhöz. 200 °C-on a foláttartalom mindössze 5 perc alatt az egyharmadára csökkent, ami azt a következtetést engedi levonni, hogy a magasabb hőmérsékletnek kitett speciális maláták nem jó folátforrások.

A táplálkozás szempontjából értékelve az eredményeket megállapítható, hogy az árpa foláttartalma csíráztatással akár tízszeresére is növelhető, amely kíméletes aszalással megőrizhető. Aszalásnál fontos, hogy a hőmérséklet ne haladja meg a 100 °C-ot. A foláttartalmat tekintve a világos árpamaláta a legjobb választás az élelmiszeripari tovább feldolgozáshoz.

A **sörfőzés** foláttartalomra gyakorolt hatásának tanulmányozásához laboratóriumi és félüzemi méretben előállított sörlevek készítése közben vettem mintákat.

A cefrézés során a növelt időtartamú fehérjepihenő nem volt hatással a foláttartalomra. A cefre foláttartalma folyamatosan nőtt, ami az enzimatikus folyamatoknak köszönhető. Az első enzimpihenő (proteáz pihenő) végére a folát jelentős része már kioldódott a malátából. A sörlé elválasztása során kiemelkedő jelentőségű a máslás, amely foláttartalma extrakt-tartalomra vonatkoztatva meghaladta a színléét. A komlóforralás, amelynél a foláttartalom csökkenésére számítani lehetett volna, nem okozott csökkenést sem laboratóriumi, sem félüzemi léptékben.

A sörfőzési folyamattal hatékonyan tudjuk kinyerni a malátából a foláttartalmat, az így előállított italok jelentős mértékben hozzájárulhatnak a napi folátbevitelhez. Eredményeim alapján megállapítható, hogy a megfelelő alapanyagok kiválasztása esetén jelentős foláttartalmú malátaitalok vagy alkoholmentes sörök állíthatóak elő. Másrészt, mivel az élesztő anyagcseréje nagymértékben befolyásolja a foláttartalom alakulását, ezért további vizsgálatokra lenne szükség normál alkoholtartalmú sörök előállítása közben, amelyek konkrétan az erjesztés során lejátszódó folyamatok hatását vizsgálják a foláttartalomra.

Az antioxidáns aktivitás és polifenol-tartalom vizsgálata a malátázás és sörfőzés során

A disszertációmban a folátok mellett az antioxidáns vegyületek a másik vizsgált csoport az egészségvédő komponenseken belül. Ez az elmúlt húsz évben kiemelt téma volt, a sörfőzés és a malátázás lépései során is széles körben tanulmányozták az antioxidáns vegyületeket, de egy átfogó kutatás, amely az alapanyagoktól a malátázás és sörfőzés technológiai lépésein át a

késztermékig vizsgálja az antioxidáns vegyületeket értékesen hozzájárulhat a meglévő ismeretekhez. A polifenolok és antioxidánsok kémiai tulajdonságaik és különösen sokszínű kémiai reakciókban való részvételük miatt közvetlenül vagy közvetve befolyásolják a sörfőzés lépéseit. A polifenolok sokféle vegyületet képezhetnek. A sörfőzés során a monomer és az oligomer polifenolok kölcsönhatásba léphetnek egymással és más vegyületekkel is mind az aerob, mind az anaerob fázisban.

A folát kísérletekhez hasonlóan először a különböző gabonákból előállított **maláták** antioxidáns aktivitását és polifenol összetételét, majd ezeknek a vegyületeknek a változását vizsgáltam félüzemi és ipari léptékű malátázás során, majd pörkölési kísérletek során.

Antioxidáns aktivitás és polifenol-tartalom szempontjából az árpamaláta kiemelkedő jelentőségű a különböző gabonafélék körében, azonban nem a világos árpamaláta, hanem a speciális karamell és festő maláta, elsősorban nagyon magas flavanol-származék tartalmuk miatt.

A polifenol-tartalmat tekintve fajta szerint a tavaszi árpák koncentrációja volt magasabb, évjárat szerint a 2018-as árpák koncentrációja volt magasabb a 2017. évi terméshez képest.

A félüzemi és nagyüzemi léptékben előállított maláták antioxidáns aktivitása és polifenol-tartalma a csírázás 3-5. napján érte el a legmagasabb értéket, majd az aszalás során csökkent. A pörkölés eredményeként az antioxidáns aktivitás minden esetben nőtt, csakúgy, mint az összes polifenol-tartalom, ami jól tükrözi a speciális malátákkal végzett előzetes kísérlet eredményeit. A hidroxi-fahéjsav-származékok koncentrációja intenzíven növekedni kezdett 150 °C körül, a flavanol-származék tartalom pedig 175 °C körül. A flavanol-származék tartalom folyamatosan nőtt, egészen a legmagasabb hőmérsékleten leghosszabb ideig történő pörkölésig, míg a többi polifenol származék koncentrációja stagnált a kezdeti növekedés után.

A magas hőmérsékleten **pörkölt maláták** kiemelkedőek, ami az antioxidáns aktivitású reduktonok képződésének és a fenolos vegyületek szerkezeti átalakulásainak köszönhető, de ez technológiai szempontból negatív lehet, mivel a sötét maláták nagy koncentrációban tartalmaznak flavanol-származékokat, amelyek képesek polimereket alkotni, ezáltal a szűrt sörök esetében hátrányosan befolyásolják a végtermék kolloid stabilitását, azonban különösen előnyösek lehetnek zavaros sörök előállítása szempontjából. Ezenkívül a magasabb hőmérsékleten kezelt maláták antioxidáns aktivitása magasabb, ami javíthatja a sörök oxidatív stabilitását.

<u>Komlók antioxidáns aktivitásának és polifenol tartalmának vizsgálata</u>

A komló a sörfőzés másik fontos alapanyaga a maláta mellett antioxidáns aktivitás és polifenol tartalom szempontjából. Magasabb polifenol tartalmú, mint az árpamaláta (árpamaláta 50-100

mg/100 g, komló legfeljebb 4 g/100 g), de a hagyományos sörök összes polifenol tartalmának csak 20–30% -át adja. A komlótobozok 3-6%-ban tartalmaznak polifenol-vegyületeket szárazanyagra vonatkoztatva.

A vizsgálatban 16 különböző típusú és alfa-sav tartalmú komló vett részt.

Nagy különbségeket találtam a különböző komlók között mind az antioxidáns aktivitás, mind a polifenol tartalom tekintetében. Nem találtam összefüggést az antioxidáns aktivitás, a polifenol tartalom és az alfa-sav tartalom között. Megállapítható, hogy a komló antioxidáns aktivitása és polifenol tartalma magas, azonban polifenol összetételben jelentős különbségek vannak. A sörfőzéshez felhasználni kívánt komló esetében fontos szem előtt tartani az előállítandó végtermék minőségi követelményeit. Ha szűretlen, zavaros terméket kívánunk előállítani, akkor megfelelő választás lehet egy magas flavanol-származék tartalmú komló, ha egy szűrt terméket készítenénk, akkor az alacsonyabb flavanol-származék koncentrációjú komló lehet a megfelelő választás.

Antioxidáns aktivitás és polifenol tartalom vizsgálata a sörfőzés folyamán

A sörfőzési kísérletekhez laboratóriumi szinten világos és sötét sörleveket, míg félüzemi szinten világos és sötét söröket állítottam elő.

A cefrézés során a világos és sötét laboratóriumi és félüzemi mintákban eltérő mértékű változás következett be, azonban az antioxidáns aktivitás és a polifenol tartalom minden esetben nőtt. A szűrés minden esetben negatív hatást gyakorolt mind az antioxidáns aktivitásra, mind a polifenol tartalomra. Fontos kihangsúlyozni, hogy a máslás magas értékeket mutatott mind antioxidáns aktivitás, mind polifenol tartalom esetében extrakt tartalomra vonatkoztatva a folát eredményekhez hasonlóan. A komlóforralás a polifenol tartalom csökkenését okozta a világos sörlevek esetében, de a sötét sörlevek forralása közben növekedést tapasztaltam. Ennek oka lehet a speciális malátákból származó reduktonok védőhatása. Az erjedés során a flavanol-származék tartalom esetében tapasztaltam a legnagyobb csökkenést, ami szűrt sör előállítása esetén pozitív lehet a végtermék kolloid stabilitása szempontjából.

A felhasznált sötét maláták magasabb antioxidáns aktivitása és polifenol tartalma a sörfőzés folyamatában is érvényesül. A késztermék antioxidáns aktivitását és polifenol összetételét nagyban befolyásolhatja a különböző típusú speciális maláták alkalmazása.

A pótanyagok hatása kongresszusi sörlevek antioxidáns aktivitására és polifenol tartalmára

A sörpiacon jelen lévő versenyhelyzet miatt a sörfőzdék nyomás alatt vannak, hogy folyamatosan magas minőségű sört állítsanak elő egyre alacsonyabb költségekkel. E cél elérése

érdekében bizonyos mértékben lecserélték a malátát különféle olcsóbb alapanyagokra, pótanyagokra. Becslések szerint világszerte a sörök 85-90% -át pótanyagok felhasználásával állítják elő. A leggyakrabban használt pótanyagok a kukorica (az összes pótanyag 46% -a), a rizs (31%), az árpa (1%), a búza (> 1%), valamint a cukrok és szirupok (22%).

A pótanyagok antioxidáns aktivitásra és polifenol összetételre gyakorolt hatásának tanulmányozása céljából laboratóriumi cefréző berendezésen kongresszusi sörleveket készítettem.

A pótanyagként alkalmazott rizs, kukorica és búza csökkentette a sörlé szabad fenolos sav tartalmát. Ez mind táplálkozási, mind technológiai szempontból negatív, mert ezek a vegyületek hozzájárulhatnak a termék hosszabb eltarthatóságához és az antioxidánsok/szabad gyökök egyensúlyának fenntartásához testünkben. Másrészt ezen pótanyagok alkalmazásakor a procianidin B3 koncentrációja csökkent, ami előnyös a késztermék jobb kolloid stabilitása szempontjából. Az árpa alkalmazása magasabb fenolos sav tartalmat eredményezett, de technológiai szempontból meg kell jegyezni, hogy flavanol tartalma (katechin, epikatechin, procianidin B3) is magas volt, ami kolloid stabilitási problémát okozhat. A speciális maláták felhasználásával készült sörlevek magas flavanol tartalommal rendelkeztek, ami összhangban van a korábbi eredményekkel. A magas flavanol tartalom elsősorban a monomereknek (katechin, epicatechin) és nem a dimereknek (procyanidin B3) köszönhető.

<u>Az infúziós és dekokciós cefrézési technológia antioxidáns aktivitásra és polifenol tartalomra gyakorolt hatásának vizsgálata</u>

Az idő folyamán számos különféle cefrézési technológia alakult ki, amelyek általában infúziós és dekokciós módszerekre oszthatók. Az infúziós technológia során a cefre teljes mennyiségének hőmérséklete folyamatosan emelkedik, az enzimatikus pihenőket bizonyos hőmérsékleteken megtartva. A másik a dekokciós, ahol a cefre egy részét elválasztják és felforralják, majd visszavezetik a másik részhez. Az infúziós (enzimatikus feltárás) vagy a dekokciós (enzimatikus és fizikai feltárás) cefrézési technológia alkalmazása nagymértékben kihat a késztermék tulajdonságaira.

Az infúziós és a dekokciós cefrézés antioxidáns aktivitásra és polifenol tartalomra gyakorolt hatásának tanulmányozása céljából egy infúziós cefrézéssel és két dekokciós cefrézéssel készült sörlevet állítottam elő félüzemi szinten.

A színlé, a máslás és a teleüst minták közül a legtöbb esetben a máslás antioxidáns aktivitása és polifenol tartalma volt a legmagasabb extrakt tartalomra vonatkoztatva, kiemelve ismét e technológiai lépés fontosságát az antioxidáns aktivitás és a polifenol tartalom szempontjából. A

dekokciós cefrézési technológia hozzájárult a sörlevek magasabb antioxidáns aktivitásához, de ez nem mondható el a polifenol tartalomra vonatkozóan.

A töménysör főzés hatása az antioxidáns aktivitásra és a polifenol tartalomra

Gazdaságossági szempontokat figyelembe véve ma a legtöbb nagy sörfőzde bizonyos termékeit úgynevezett töménysör főzésével állítja elő. Ez azt jelenti, hogy a főzőházban koncentráltabb sörlét főznek, mint ami a késztermék extrakt tartalma lesz, majd az erjedés után ezt a tömény sört általában a szűrés során hígítják a késztermék paramétereinek megfelelően.

Ennek a tanulmányozásához két sört készítettem ugyanazokból az alapanyagokból és ugyanazzal a technológiával félüzemi méretben. Az egyiket 9 %m/m, a másikat 14 %m/m eredeti extrakt tartalommal. A laboratóriumi mérések elvégzése előtt a 14% -os sört oxigénmentes vízzel 9% -ra hígítottam.

Nem figyelhető meg szignifikáns különbség a két minta antioxidáns aktivitása között, másrészt az összes vizsgált polifenol vegyület koncentrációja szignifikánsan magasabb volt a hagyományos eljárással készített sörben, mint a töménysörként készített, majd hígított mintában. A töménysör főzés csökkentette a sör polifenol tartalmát, ami rosszabb ízstabilitást és kevésbé telt ízt eredményezhet, de a termék kolloid stabilitása jobb lehet.

10. APPENDICES
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